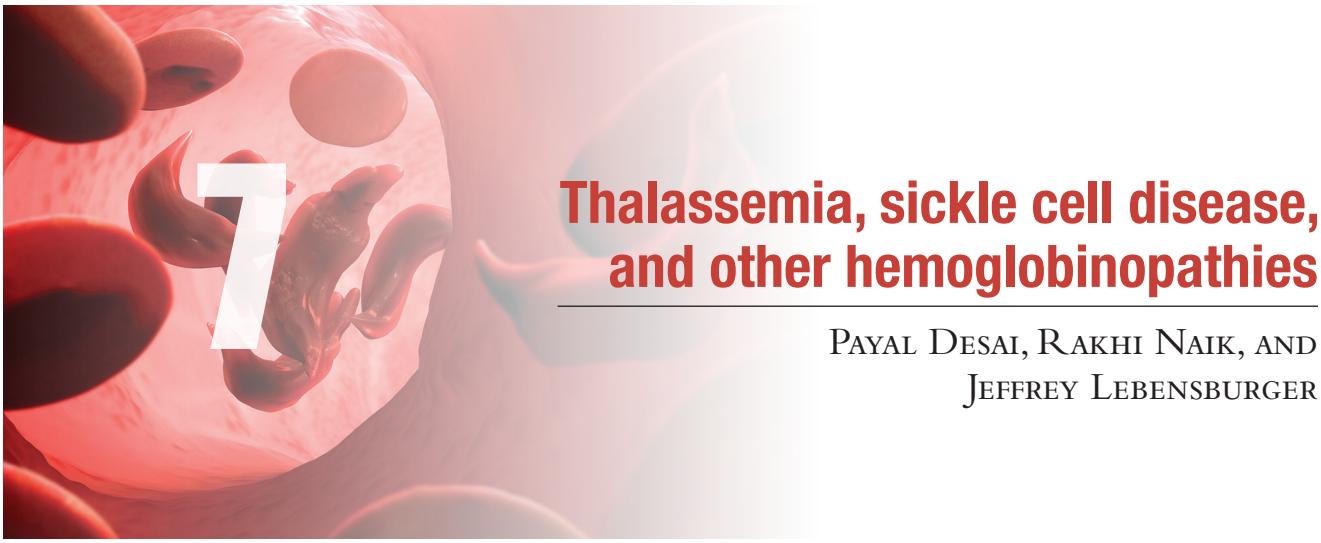

7 Thalassemia, sickle cell disease, and other hemoglobinopathies

Payal Desai, Rakhi Naik, and Jeffrey Lebensburger

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The online version of this chapter contains an educational multimedia component on sickle cell disease therapies.

Introduction

Hemoglobin (Hb) is the oxygen-carrying protein within red blood cells (RBCs). It is composed of 4 globular protein subunits, called globins, each with an oxygen-binding heme group. The 2 main types of globins are the α-globins and the β-globins, which are made in essentially equivalent amounts in precursors of RBCs. Normal adult Hb (HbA) has 2 α-globins and 2 β-globins ($\alpha_2\beta_2$). Genes on chromosomes 16 and 11 encode the α-globins and β-globins, respectively. There are also distinct embryonic, fetal, and minor adult analogues of the α-globins and β-globins encoded by separate genes.

Hemoglobin structure

Hemoglobin is a tetramer consisting of 2 pairs of globin chains. Heme, a complex of ferrous iron and protoporphyrin, is linked covalently to each globin monomer and can reversibly bind 1 oxygen molecule. The molecular mass of Hb is approximately 64 kDa. The α-chains contain 141 amino acids, and the β-chains contain 146 amino acids, as do the β-like globins, δ and γ, which differ from β by 10 and 39 amino acids, respectively. The compositions of normal Hb species throughout development are depicted in Figure 7-1. The postembryonic Hbs are HbA ($\alpha_2\beta_2$), HbA_2 ($\alpha_2\delta_2$), and HbF ($\alpha_2\gamma_2$).

When Hb is deoxygenated, there are substantial changes in the structures of the individual globins and the Hb tetramer. The iron molecule rises from the plane of its heme ring, and there is a significant rotation of each globin chain relative to the others. In the deoxy conformation, the distance between the heme moieties of the β-chains increases by 0.7 nm. This conformation is stabilized in a taut (T) conformation by salt bonds within and between globin chains and by the binding of allosteric modifiers such as 2,3-bisphosphoglycerate (2,3-BPG) and of protons. The binding of oxygen to Hb leads to disruption of the salt bonds and transition to a relaxed (R) conformation.

Hemoglobin function

Hemoglobin enables RBCs to deliver oxygen to tissues by its reversible binding of oxygen. With the sequential binding of 1 oxygen molecule to each of the 4 heme groups, the salt bonds are progressively broken, which increases the oxygen affinity of the remaining heme moieties. Cooperation between the heme

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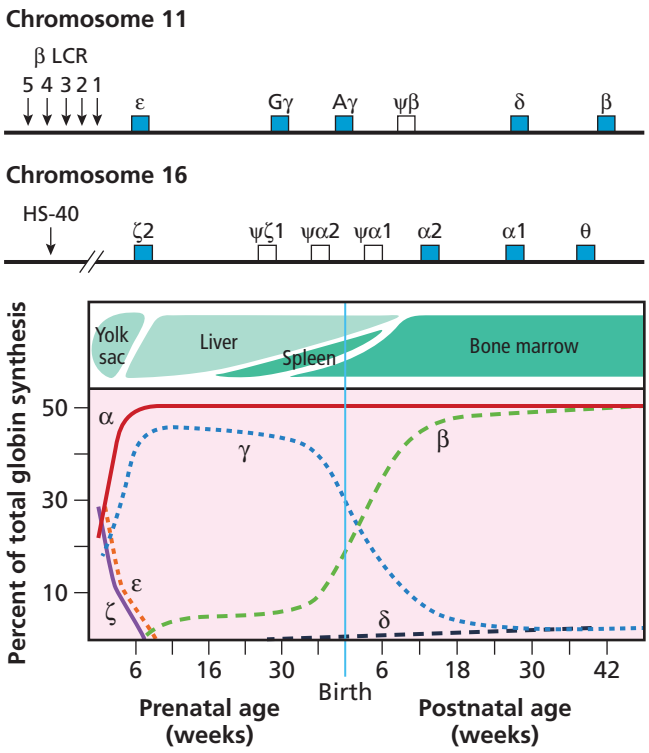

Figure 7-1 Hemoglobin gene clusters and developmental hematopoiesis. The organization of the α- and β-globin gene clusters are shown at the top of the figure. The bottom portion of the figure illustrates the developmental changes in Hb production, both by the site of production of blood and changes in the proportions of the different globins. HS-40, DNAse I hypersensitive site; LCR, locus control region. Adapted from Stamatoyannopoulos G et al, eds, The Molecular Basis of Blood Diseases, 3rd ed. (WB Saunders; 2001), with permission.

rings results in the characteristic sigmoid-shaped oxygen affinity curve. This phenomenon accounts for the release of relatively large amounts of oxygen with small decreases in oxygen tension.

Deoxygenation of Hb is modulated by certain biochemical influences. For example, deoxyhemoglobin binds protons with greater avidity than oxyhemoglobin, which results in a direct dependence of oxygen affinity on pH over the physiologic pH range. This Bohr effect has 2 major physiologic benefits: (1) the lower pH of metabolically active tissue decreases oxygen affinity, which accommodates oxygen delivery; and (2) the higher pH level resulting from carbon dioxide elimination in the lungs increases oxygen affinity and oxygen loading of RBCs. An additional important influence on oxyhemoglobin dissociation is temperature. Hyperthermia decreases affinity, providing the opportunity to deliver more oxygen at the tissue level. 2,3-BPG, a metabolic intermediate of anaerobic glycolysis, is another physiologically important modulator of oxygen affinity. When 2,3-BPG binds in the pocket formed by the amino termini of the β-chains, it stabilizes the deoxy conformation of Hb, thereby reducing its oxygen affinity. The intraerythrocytic molar concentrations of 2,3-BPG and Hb are normally about equal (5 mM). When 2,3-BPG levels increase during periods of hypoxia, anemia, or tissue hypoperfusion, oxygen unloading to tissues is enhanced.

Carbon dioxide reacts with certain amino acid residues in the β-chain of Hb; however, this does not play a significant role in carbon dioxide transport. It recently has been reported that Hb binds nitric oxide in a reversible manner. The participation of Hb in modifying regional vascular resistance through this mechanism has been proposed.

Hemoglobin production

The α-globin gene cluster is on chromosome 16 and includes the embryonic ζ-globin gene and the duplicated α-globin genes (α_1 and α_2), which are expressed in both fetal and adult life. The β-globin gene cluster is on chromosome 11 and includes an embryonic ε-globin gene, the 2 fetal γ-globin genes (Aγ and Gγ), and the 2 adult δ- and β-globin genes. Both clusters also contain nonfunctional genes (pseudogenes) designated by the prefix ψ. The θ-globin gene downstream of α_1 has unknown functional significance.

The expression of each globin gene cluster is under the regulatory influence of a distant upstream locus control region (LCR). The LCR for the β-cluster resides several kilobases upstream. A similar regulatory region, called HS-40, exists upstream of the α cluster. The LCRs contain DNA sequence elements that interact with erythroid-specific and nonspecific DNA-binding proteins. LCRs serve as a "master switch" by inducing expression of downstream gene clusters. LCRs also facilitate the binding and interaction of transcriptional regulatory proteins in proximity to the specific genes within the downstream cluster. These regulatory proteins influence the promoter function of the α-globin and β-globin genes to achieve a high level of erythroid- and development-specific gene expression.

Figure 7-1 details the organization of the α- and β-clusters with the associated upstream regulatory elements and the normal Hb species produced during the developmental stages from intrauterine to adult life. Note that the genes are expressed developmentally in the same sequence in which they are organized physically in these clusters (left to right, 5′ to 3′). The process of developmental changes in the type and site of globin gene expression is known as *Hb switching*. Switching

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within the cluster is influenced by differential enhancing and gene-silencing effects imparted by the combination of the LCR and local regulatory proteins, but the entire process of regulatory determination remains incompletely defined. The ability to modulate the switch from the synthesis of γ- to β-globin chains has long been of interest because "reversing the switch" to enhance expression of fetal hemoglobin (HbF) could potentially successfully treat sickle cell disease (SCD). A number of different transcription factors have been identified, including MYB, KLFs, BCL11A, and SOX6, which repress fetal globin gene expression in erythroid cells. Turning off these repressors could increase HbF expression and treat hemoglobinopathies.

Disorders of hemoglobin

Disorders of hemoglobin can be classified as quantitative or qualitative. Quantitative Hb disorders result from the decreased and imbalanced production of generally structurally normal globins. For example, if β-globin production is diminished by a mutation, there is a relative excess of α-globin chains. Such imbalanced production of α- and β-globin chains damages RBCs and their precursors in the bone marrow. These quantitative Hb disorders are called thalassemias. Qualitative abnormalities of Hb arise from mutations that change the amino acid sequence of the globin, thereby producing structural and functional changes in Hb. There are 4 ways in which Hb can be qualitatively abnormal: (1) decreased solubility (eg, sickle hemoglobin [HbS]), (2) instability (eg, Hb Köln), (3) altered oxygen affinity (eg, HbM-Saskatoon), and (4) altered maintenance of the oxidation state of the heme-coordinated iron (eg, HbM-Iwate).

Hemolysis is the accelerated destruction of RBCs leading to decreased RBC survival. The bone marrow's response to hemolysis is increased erythropoiesis, reflected by reticulocytosis. As is typical in hemoglobinopathies, the bone marrow is unable to completely compensate for hemolysis, leading to anemia. In hemoglobinopathies, hemolytic anemia results from decreased solubility and instability of Hb.

Qualitative hemoglobin disorders often are referred to as *hemoglobinopathies,* even though the term technically can apply to both qualitative and quantitative disorders. Both qualitative and quantitative disorders of Hb can be subdivided by the particular globin that is affected; for example, there are α-thalassemias and β-hemoglobinopathies, among others. We begin this chapter with a review of the thalassemias and end the section with a discussion of several of the common qualitative Hb disorders.

Thalassemia

CLINICAL CASE

A healthy 48-year-old female of African descent is referred to you for evaluation of refractory microcytic anemia. She has been treated with oral iron formulations many times throughout her life. Hemoglobin values have always ranged from 10 to 11 g/dL with a mean corpuscular volume (MCV) ranging from 69 to 74 fL. She has no other prior medical history. Her examination is entirely unremarkable. Peripheral blood smear is significant for microcytosis, mild anisopoikilocytosis, and a small number of target cells. The Hb concentration is 10 g/dL with an MCV of 71 fL and mean corpuscular hemoglobin (MCH) of 23 pg. Additional laboratory studies include a transferrin saturation of 32% and a normal ferritin of 285 ng/mL. Hemoglobin electrophoresis reveals HbA 98% and HbA2 1.8%.

Thalassemia occurs when there is quantitatively decreased synthesis of often structurally normal globin proteins. Mutations that decrease the synthesis of α-globins cause α-thalassemia; mutations that decrease the synthesis of β-globins cause β-thalassemia. In general, α-thalassemias are caused by deletions of DNA, whereas β-thalassemias are caused by point mutations. Heterozygous thalassemia (thalassemia trait) appears to confer protection against severe *Plasmodium falciparum* malarial infection. As a result of this selective advantage, a wide variety of independent mutations leading to thalassemia have arisen over time and have been selected for in populations residing in areas where malaria is (or once was) endemic. The major result of a deletion or mutation in all forms of thalassemia is decreased or absent production of 1 or more globin chains. This results in unbalanced synthesis of individual alpha and beta subunits. Unpaired α- or β-globin chains are insoluble or form tetramers that do not release oxygen readily and precipitate in the red cells (eg, α4, β4). For example, if β-globin synthesis is reduced by a mutation, there is a relative excess of α-globin chains. Such imbalanced production of α- and β-globin chains results in damage to RBC precursors in the bone marrow. This damage occurs largely because the excess unpaired globin is unstable, and precipitates within early RBC precursors in the bone marrow and oxidatively damages the cellular membrane. If the α- and β-globin imbalance is severe, most of the RBC precursors in the bone marrow are destroyed before they can be released into the circulation. A severe microcytic anemia results. The body attempts to compensate for the anemia by increasing erythropoietic activity throughout the marrow and sometimes in extramedullary spaces, although this effort is inadequate and compensation is incomplete.

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This pathophysiologic process is called ineffective erythropoiesis. The complications of thalassemia vary and depend on the severity of the chain imbalance and identity of the globin chain.

Clinical classification of thalassemia

In the past, thalassemias were described using 2 independent nomenclatures: genetic and clinical. The genetic nomenclature denotes the type of causative mutation, such as α-thalassemia or β-thalassemia. The clinical nomenclature divides the thalassemias into the asymptomatic trait state (thalassemia minor), severe transfusion-dependent anemia (thalassemia major), and everything in between (thalassemia intermedia). The 2 nomenclature systems can be used together, for example, α-thalassemia minor or β-thalassemia intermedia. A new clinical classification system for thalassemia has been adopted by the Thalassemia International Federation in its recent guidelines and categorizes thalassemia into transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT) categories, which include various genotypes affecting the α- or β-globin genes, and hemoglobinopathies including hemoglobin E. Categorization into either TDT or NTDT involves a thorough clinical evaluation, including clinical symptoms of anemia, severity of anemia, signs of extramedullary hematopoiesis, and transfusion requirements (Figures 7-2 and 7-3).

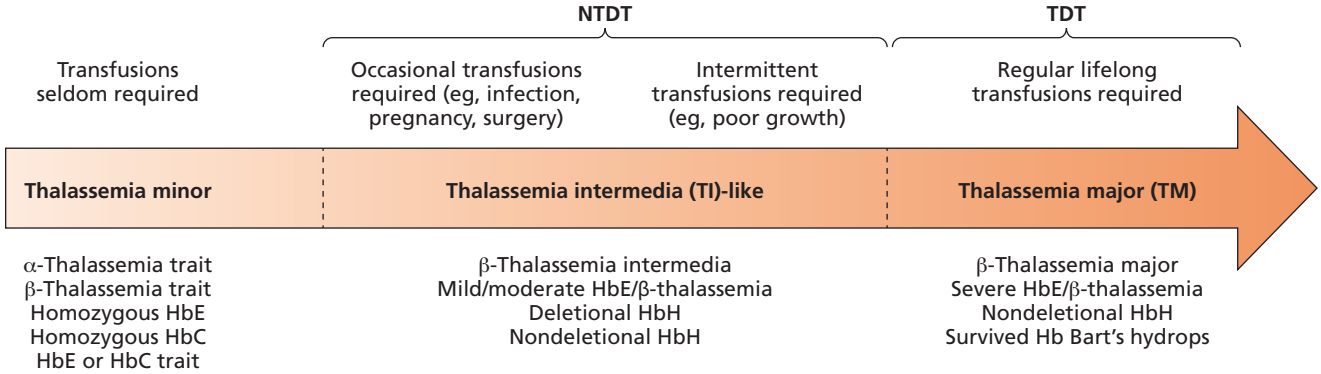
β-Thalassemias

β-Thalassemia is prevalent in the populations where malaria was once endemic, such as the Mediterranean region, the Middle East, India, Pakistan, and Southeast Asia, and is somewhat less common in Africa. It is rarely encountered in Northern European White persons. However, because of migration, thalassemia (both α- and β-thalassemia) is now found in most regions of the world, including North America.

Molecular basis

β-Thalassemia results from >350 different mutations in the β-globin gene complex (Figure 7-4). Abnormalities have been identified in the promoter region, mRNA cap site, 5′ untranslated region, splice sites, exons, introns, and polyadenylation signal region of the β-globin gene. Gene deletions are infrequent except in δβ- and εγδβ-thalassemias. A variety of single base-pair mutations or insertions or deletions of nucleotides represent the majority of described mutations. Thus, defects in transcription, RNA processing, and translation or stability of the β-globin gene product have been observed. Mutations within the coding region of the globin gene allele may result in nonsense or truncation mutations of the corresponding globin chain, leading to complete loss of globin synthesis from that allele (β^0-thalassemia allele). Alternatively, abnormalities of transcriptional regulation or mutations that alter splicing may cause mild to markedly decreased, but not absent, globin gene synthesis (β^+-thalassemia allele). β-thalassemia major (Cooley's anemia) and β-thalassemia intermedia can caused by various genotypes, including homozygosity or compound heterozygosity for 2 β^0 alleles (β^0/β^0) or compound heterozygosity with a β^0 and β^+ allele (β^0/β^+). Patients with β-thalassemia traits are generally heterozygous, carrying a single β-thalassemia allele (β/β^0, β/β^+), but some patients who are homozygous or compound heterozygous for 2 very mild β^+ alleles may also have a β-thalassemia minor phenotype. The clinical phenotype of patients with β-thalassemia is heterogeneous and is determined primarily by the globin chain imbalance because of the number and severity of the abnormal alleles inherited. Coinheritance of other abnormalities affecting α- or δ-globin synthesis or structural abnormalities of

Figure 7-2 The clinical spectrum of thalassemia syndromes based on transfusion requirement. Hb, hemoglobin; NTDT, non-transfusion-dependent thalassemia; TDT, transfusion-dependent thalassemia. Adapted from Musallam KM et al, *Haematologica*. 2013;98(6):833-844, with permission.



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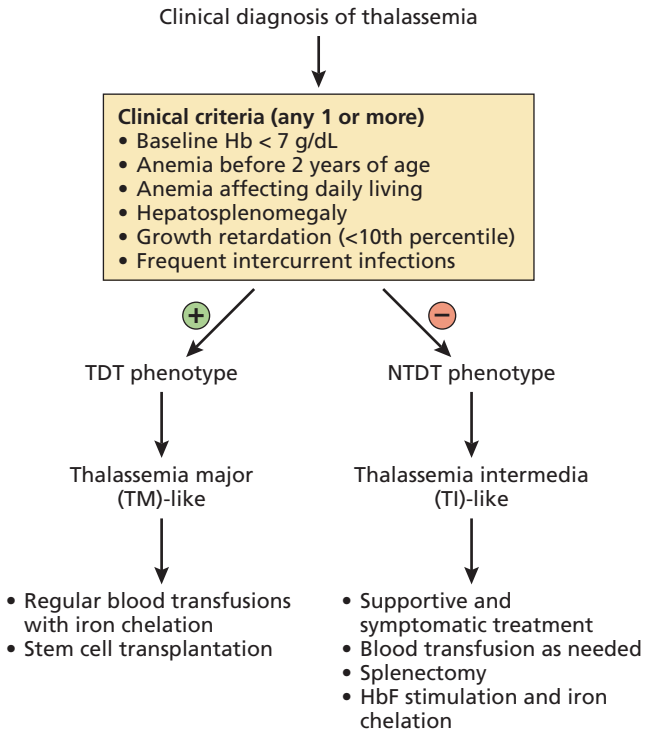
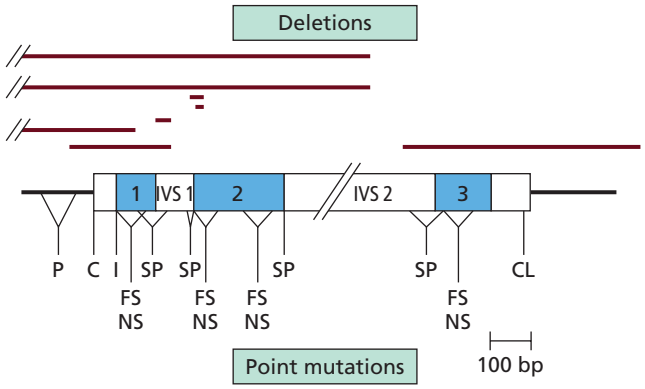

Clinical diagnosis of thalassemia
Clinical criteria (any 1 or more)
• Baseline Hb < 7 g/dL
• Anemia before 2 years of age
• Anemia affecting daily living
• Hepatosplenomegaly
• Growth retardation (<10th percentile)
• Frequent intercurrent infections
+
−
TDT phenotype
NTDT phenotype
Thalassemia major (TM)-like
Thalassemia intermedia (TI)-like
• Regular blood transfusions with iron chelation
• Stem cell transplantation
• Supportive and symptomatic treatment
• Blood transfusion as needed
• Splenectomy
• HbF stimulation and iron chelation

Figure 7-3 Diagnosis, classification, and management of transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT). Hb, hemoglobin. Adapted from Viprakasit V, Ekwattanakit S, *Hematol Oncol Clin N Am*. 2018;32(2):193-211 (© 2018), with permission from Elsevier.

Hb (eg, HbC, HbE) also affects the chain imbalance and thus the clinical phenotype. For example, patients with β/β^0 or β/β^+ mutations may present with a phenotype of β-thalassemia intermedia if alpha triplication or quadruplication is present, leading to further imbalance in the alpha-to-beta globin ratio. Secondary genetic modifiers, such as uridine diphosphate glucuronosyltransferase gene polymorphisms, also contribute to the overall phenotype.

Figure 7-4 Common β-thalassemia mutations. The major classes and locations of mutations that cause β-thalassemia are shown. C, cap site; CL, RNA cleavage [poly(A)] site; FS, frameshift; I, initiation codon; IVS, intervening sequence; NS, nonsense; P, promoter boxes; SP, splice junction, consensus sequence, or cryptic splice site. Adapted from Stamatoyannopoulos G et al, eds, The Molecular Basis of Blood Diseases, 3rd ed. (WB Saunders; 2001), with permission.



Pathophysiology

The defect in β-thalassemia is a reduced or absent production of β-globin chains with a relative excess of α-chains. The decreased β-chain synthesis leads to impaired production of the $\alpha_2\beta_2$ tetramer of HbA, decreased Hb production, and an imbalance in globin chain synthesis. The reduction in HbA in each of the circulating RBCs results in hypochromic, microcytic RBCs with target cells, a characteristic finding in all forms of β-thalassemia. Aggregates of excess α-chains precipitate and form inclusion bodies, leading to premature destruction of erythroid precursors in the bone marrow (ineffective erythropoiesis) (Figure 7-5). In more severe forms, circulating RBCs may also contain inclusions, leading to early clearance by the spleen. The precipitated α-globin chains and products of degradation may also induce changes in RBC metabolism and membrane structure, leading to shortened RBC survival. The response to anemia and ineffective erythropoiesis is increased production of erythropoietin leading to erythroid hyperplasia, which can produce skeletal abnormalities, splenomegaly, extramedullary masses, and osteoporosis. Ineffective erythropoiesis is associated with increased gastrointestinal iron absorption because of decreased hepcidin. RBC membrane damage with increased surface expression of anionic phospholipids, platelet activation, and changes in hemostatic regulatory proteins contribute to a hypercoagulable state in thalassemia, which is worsened after splenectomy.

β-Thalassemia trait

β-Thalassemia trait (minor) is asymptomatic and is characterized by mild microcytic anemia. Most commonly, it arises from heterozygous β-thalassemia (β-thalassemia trait). Neonates with β-thalassemia trait have no anemia or microcytosis; these develop with increasing age as the transition from HbF to HbA production progresses. Patients with β-thalassemia trait may have a Hb ranging from 9 g/dL to a normal value. Peripheral smear shows microcytic, hypochromic RBCs, poikilocytes, and target cells. Basophilic stippling is variable. The MCV is usually <70 fL, the MCH is reduced (MCH <26 pg), and the reticulocyte count can be mildly elevated. HbA_2 levels are diagnostically elevated to >3.5% (usually 4% to 7%), and HbF levels may be mildly increased. RBC survival is normal, with minimal ineffective erythropoiesis. Individuals with β-thalassemia trait are asymptomatic and do not

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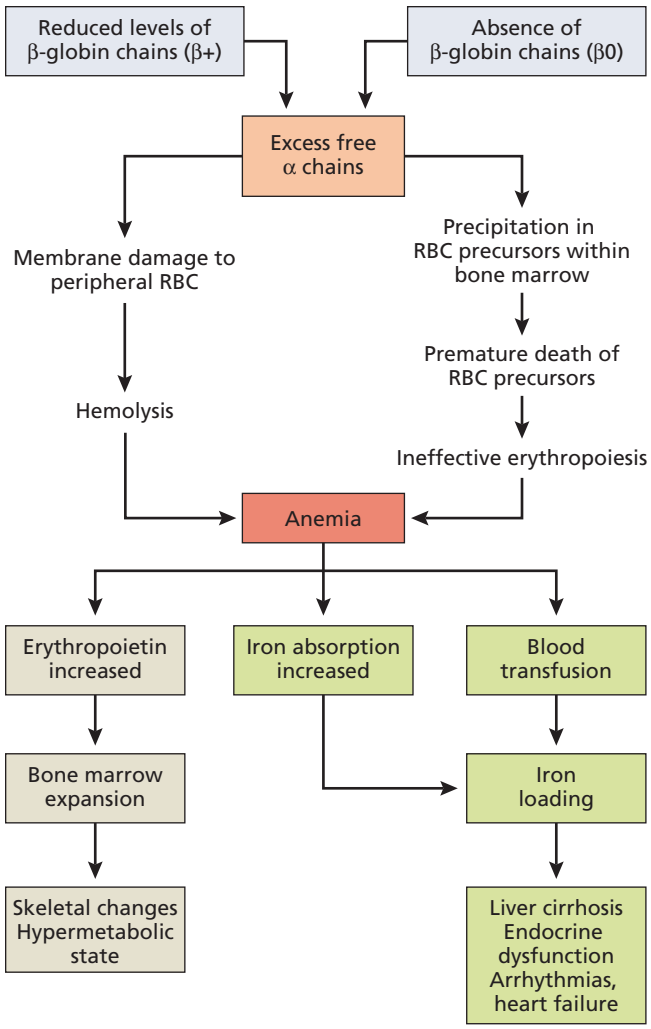

Figure 7-5 Pathophysiology of β-thalassemia. Effects of excess production of free α-globin chains in β-thalassemia. RBC, red blood cell. Adapted from Viprakasit V, Origa R, in: Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT), 3rd ed. (Thalassaemia International Federation; 2014), with permission. Additional reproduction is prohibited.

require therapy. They should be identified to reduce the risk of inappropriate iron supplementation. Individuals of childbearing age should be offered genetic counseling for informed reproductive choices.

Transfusion-dependent β-thalassemia

Patients with TDT require regular blood transfusions for survival. Two important TDT syndromes are β-thalassemia major syndromes (homozygous β^0-thalassemia) and compound heterozygote states such as severe HbE/β-thalassemia,

β-Thalassemia major (Cooley anemia) is characterized by absence of or severe deficiency in β-chain synthesis. Symptoms are usually evident within the first 6 to 12 months of life as the HbF level begins to decline and severe anemia occurs with Hb <7 g/dL. In the absence of adequate RBC transfusions, the infant presents with pallor, irritability, jaundice, failure to thrive, and a variety of clinical findings. Erythroid expansion leads to facial deformities, including frontal bossing and maxillary prominence. Increased erythroid expansion widens the bone marrow space, thins out the cortex, and causes low bone density, which may predispose some patients with TDT to fractures. Growth retardation, progressive hepatosplenomegaly, gallstone formation, and cardiac disease are common. Most homozygotes do not survive without transfusions beyond the age of 5 years. RBC transfusions ameliorate severe anemia and suppress ineffective erythropoiesis.

A child with β-thalassemia major who is not receiving transfusions suffers from severe anemia. Peripheral blood smear findings include anisopoikilocytosis, target cells, severe hypochromia, nucleated red blood cells, and basophilic stippling. The reticulocyte count is slightly increased, and nucleated RBCs are abundant. These findings are exaggerated after splenectomy. Hemoglobin electrophoresis reveals persistent elevation of HbF ($\alpha_2\gamma_2$) and variable elevation of HbA_2 ($\alpha_2\delta_2$). HbA is absent in homozygous β^0-thalassemia.

Non-transfusion-dependent β-thalassemia

Non-transfusion-dependent thalassemia includes a wide spectrum of clinical phenotypes, ranging from mild to moderately severe anemia. Patients with NTDT do not require regular blood transfusions for survival. Intermittent transfusions may be required in acutely worsening anemia caused by infection or acute illness, or to allow for normal growth and development in childhood. Some patients with NTDT may require regular transfusions later on in life, often in adulthood, because of complications of the disease including worsening anemia and splenomegaly. The β-thalassemia NTDT encompasses 2 clinically distinct forms, including β-thalassemia intermedia and moderate HbE/β-thalassemia syndromes.

These patients exhibit a wide spectrum of clinical findings, from mild to more significant complications including hepatosplenomegaly, extramedullary hematopoietic pseudotumors, bone deformities, leg ulcers, delayed puberty, thrombotic events, pulmonary hypertension, silent infarcts, gallstones, and iron overload. These complications, except for iron overload, are generally limited in the well-transfused thalassemia patient because transfusion interrupts the underlying pathophysiology. Most indications for initiating a chronic transfusion program in NTDT are similar to those in TDT. However, these are generally initiated later in childhood or in adulthood, depending on the severity of the phenotype.

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Some patients may present with mild phenotypes in childhood, and subsequently develop worsening anemia, increased extramedullary hematopoiesis, and endocrine complications in adulthood that may warrant initiation of a chronic transfusion program. Thus, it is important to closely follow all individuals with NTDT long term, with regular-interval evaluation of complications (Figure 7-2 and Figure 7-3).

A variable degree of anemia with hypochromic, microcytic cells and target cells is observed in NTDT. Laboratory abnormalities are similar to β-thalassemia trait, but more severe.

α-Thalassemias

There is a high prevalence of α-thalassemia in areas of the world where malaria was once endemic, including Africa, the Mediterranean region, Southeast Asia, and, to a lesser extent, the Middle East.

Molecular basis

Two copies of the α genes are normally present on each chromosome 16, making the defects in α-thalassemia more heterogeneous than in β-thalassemia (Figure 7-6). The α^+-thalassemias result from deletion of one of the linked genes, $-\alpha/\alpha\alpha$, or impairment caused by a point mutation, designated $\alpha^T\alpha/\alpha\alpha$. The deletion type of α^+-thalassemia is caused by unequal crossover of the linked genes, whereas the nondeletion type includes mutations resulting in abnormal transcription or translation or the production of unstable α-globin. The $-\alpha/\alpha\alpha$ genotype (the "silent carrier" state) occurs in approximately 1 in 3 African Americans. Hb Constant Spring is one example of many nondeletional α-thalassemias. It is a nondeletional α^+-thalassemia, common in Southeast Asia, resulting from a mutation that affects termination of translation and results in abnormally elongated α-chains. The $--/\alpha\alpha$ genotype (α^0-thalassemia) of α-thalassemia trait because of a loss of linked α-genes on the same chromosome (*cis* configuration), is more common in individuals of Asian descent, whereas the $-\alpha/-\alpha$ genotype (deletions in the *trans* position) is more common in individuals of African or Mediterranean descent.

Pathophysiology

As in the β-thalassemias, the imbalance of globin chain synthesis results in decreased Hb synthesis and microcytic anemia. Excess γ- and β-chains form tetramers termed Hb Barts and HbH, respectively. These tetramers are more soluble than unpaired α-globins (as in β-thalassemia) and form RBC inclusions slowly. Consequently, although α-thalassemia is associated with a hemolytic anemia, it does not lead to significant ineffective erythropoiesis.

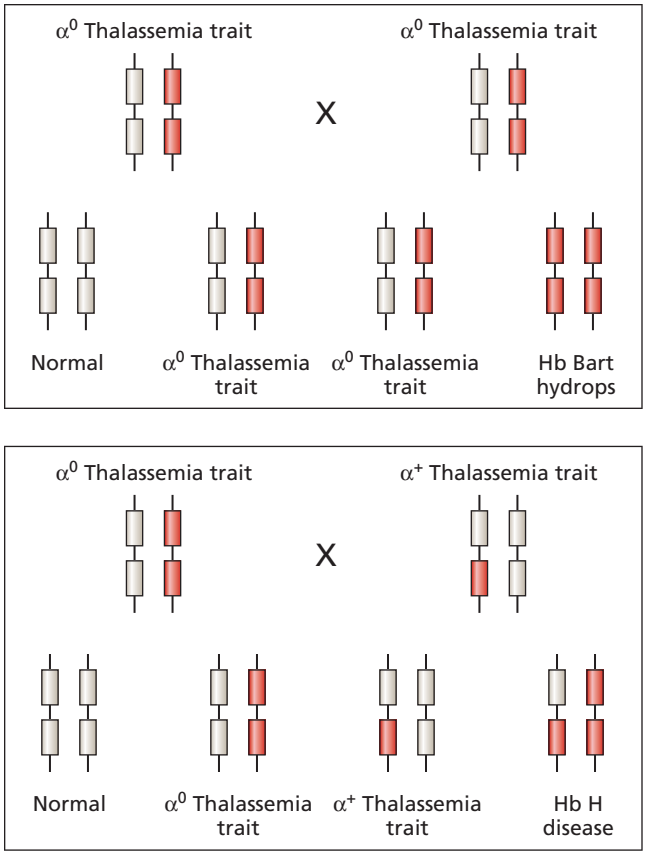

Figure 7-6 Genetics of α-thalassemia. The α-globin genes are represented as boxes. The red α-globin genes represent deletions or otherwise inactivated α-genes. The open boxes represent normal α-genes. The terms α^0- and α^+-thalassemia are defined in the text. The potential offspring of 2 parents with α^0-thalassemia trait is shown in the upper panel. The potential offspring of 1 parent with α^0-thalassemia trait and the other with α^+-thalassemia trait is shown in the lower panel (note the lack of Hb Barts hydrops fetalis in these offspring). Adapted from Stamatoyannopoulos G et al, eds, The Molecular Basis of Blood Diseases, 3rd ed. (WB Saunders; 2001), with permission.

The homozygous inheritance of α^0-thalassemia ($--/--$) results in the total absence of α-chains, death in utero, or hydrops fetalis. Unpaired γ-chains form Hb Barts (γ_4), and there may be persistence of embryonic Hbs. Hb Bart is soluble and does not precipitate; however, it has a very high oxygen affinity and is unable to deliver oxygen to the tissues. This leads to severe fetal tissue hypoxia, resulting in edema, congestive heart failure, and death. HbH disease results from the coinheritance of α^0-thalassemia and α^+-thalassemia ($--/-\alpha$) or α^0-thalassemia and a nondeletional form of α-thalassemia ($--/\alpha^T\alpha$) such as Hb Constant Spring ($--/\alpha^{CS}\alpha$). The excess β-chains form HbH (β_4) that is unstable, causing precipitation within circulating cells and hemolysis. Patients have moderately severe hemolytic anemia.

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HbH also can be produced as an acquired phenomenon in the setting of myelodysplastic syndromes and some myeloid leukemias, in which somatic mutations of the *ATRX* gene downregulate α-globin production and cause globin chain imbalance. This condition is called α-*thalassemia–myelodysplastic syndrome.* The X-linked *ATRX* gene encodes a chromatin-remodeling factor (X-linked helicase 2) that regulates α-globin production. Constitutional deletions of this gene produce the α-thalassemia X-linked intellectual disability syndrome.

α-Thalassemia trait

In contrast to β-thalassemia, α-thalassemia can manifest in both fetal and postnatal life. The clinical manifestations of α-thalassemia are related to the number of functional α-globin genes (Figure 7-6). Heterozygotes for α^+-thalassemia ($-\alpha/\alpha\alpha$), called *silent carriers,* are clinically normal with minimal to no anemia or morphologic abnormalities of RBCs. The Hb electrophoresis is normal. Thalassemia trait (2-gene deletion α-thalassemia) occurs in 2 forms: α^0-thalassemia trait ($--/\alpha\alpha$) or homozygosity for α^+-thalassemia ($-\alpha/-\alpha$). Individuals with thalassemia trait have a lifelong mild microcytic anemia. In newborns who are heterozygous for α^0-thalassemia ($--/\alpha\alpha$), Hb electrophoresis reveals 2% to 5% Hb Barts and microcytosis (MCV <95 fL). Children and adults heterozygous for α^0-thalassemia ($--/\alpha\alpha$) or homozygous for α^+-thalassemia ($-\alpha/-\alpha$) have mild anemia with hypochromic, microcytic RBCs and target cells. The RBC indices are similar to those of β-thalassemia trait, but the Hb electrophoresis is normal (HbA_2 <3.5%). Molecular testing is required to confirm the diagnosis in α-thalassemia caused by 1 or 2 gene deletions. The high prevalence of the $-\alpha/-\alpha$ genotype in African Americans is noteworthy. About 2% to 3% of all African Americans in the United States have asymptomatic microcytosis and borderline anemia (often mistaken for iron deficiency) as a result of this condition. Individuals with 1 or 2 alpha gene deletions ($-\alpha/\alpha\alpha$, $--/\alpha\alpha$, $-\alpha/-\alpha$) do not generally require any specific treatment. Individuals of at-risk ethnicities of childbearing age who are at risk of HbH disease or hydrops fetalis should be offered genetic counseling for informed reproductive choices.

Transfusion-dependent α-thalassemia

Similar to the β-thalassemia TDT syndromes, the α-thalassemia TDT syndromes result in transfusion dependence. In the absence of adequate RBC transfusions, patients develop signs/symptoms of severe anemia and sequelae of ineffective erythropoiesis such as erythroid marrow expansion and extramedullary hematopoiesis. The transfusion-dependent α-thalassemia syndromes include severe nondeletional HbH disease and those who survived Hb Barts hydrops fetalis.

Homozygous α^0-thalassemia ($--/--$) results in Hb Barts hydrops fetalis syndrome. The lack of HbF caused by the absence of α chains produces intrauterine hypoxia, resulting in marked expansion of bone marrow and hepatosplenomegaly in the fetus and enlargement of the placenta. In-utero death usually occurs between 30 and 40 weeks or soon after birth. The blood smear in Hb Barts hydrops fetalis syndrome ($--/--$) reveals markedly abnormal RBC morphology with anisopoikilocytosis, hypochromia, targets, basophilic stippling, and nucleated RBCs. The Hb electrophoresis in a neonate reveals approximately 80% Hb Barts and the remainder Hb Portland ($\zeta_2\ \gamma_2$). A fetus with homozygous α^0-thalassemia can be rescued with intrauterine transfusions, typically initiated at 24 weeks gestation and continued until term, with fetal middle cerebral artery Doppler velocity monitoring as a guide for the degree of fetal anemia. Such patients need postnatal chronic transfusions throughout life or stem cell transplantation. Maternal complications resulting from a homozygous α^0-thalassemia fetus include preeclampsia, hypertension, hemorrhage, dystocia, and retained placenta. Because of the high prevalence of the α^0 genotype in southeast Asian and certain Mediterranean populations, screening programs and genetic counseling can reduce the occurrence of births resulting in Hb Barts hydrops fetalis and HbH disease.

Non-transfusion-dependent thalassemia

The major α-thalassemia NTDT syndrome is HbH disease. The clinical manifestations are variable in HbH disease ($--/-\alpha$), with severe forms resulting in transfusion dependence, and other individuals having a milder course. As in β-thalassemia intermedia, splenomegaly occurs commonly in the anemic patient. The homozygous state for Hb Constant Spring results in moderate anemia with splenomegaly. HbH disease ($--/-\alpha$) is characterized by anisopoikilocytosis and hypochromia with elevated absolute reticulocyte counts. Hemoglobin electrophoresis reveals 5% to 40% of the rapidly migrating HbH. Supravital staining with brilliant cresyl blue reveals inclusions representing in vitro precipitation of HbH. Patients with HbH disease are categorized as NTDT and usually require no specific interventions. However, nondeletional HbH disease, such as HbH Constant Spring ($--/\alpha\ ^{CS}\alpha$) is typically more severe than classical HbH disease ($--/-\alpha$) and

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individuals often require intermittent or chronic RBC transfusions. Because the forms of thalassemia that start as NTDT at a young age may have a variable phenotype with increasing age, close observation and follow-up is important.

Clinical complications

Complications in TDTs and NTDTs affect multiple systems and are caused by chronic hemolysis, ineffective erythropoiesis, increased intestinal iron absorption, and transfusional iron overload. Management of patients with TDT and NTDT involves a comprehensive multidisciplinary care approach. Table 7-1 summarizes the difference in complications between TDT and NTDT.

Iron overload

Iron overload is a major complication in TDT and NTDT. Each milliliter of transfused blood contains 1 mg of iron. Red blood cell transfusions are the major cause of iron loading in TDT. Additionally, ineffective erythropoiesis drives iron to continue to accumulate from intestinal absorption. This accumulation of excess iron, from either the intestines or transfusion, is exacerbated as the body does not have an active mechanism to excrete excess iron aside from mucosal sloughing or bleeding. The excess iron results in increased nontransferrin-bound iron, which generates harmful reactive oxygen species leading to lipid peroxidation and organelle and DNA damage causing apoptosis, fibrosis, and organ damage. Uncontrolled transfusional iron loading leads to iron deposition in key organs leading to an increased risk of liver cirrhosis, hepatocellular carcinoma, heart failure, and endocrine complications including hypogonadotropic hypogonadism, diabetes, hypothyroidism, osteoporosis, and hypoparathyroidism. An increased frequency of *Yersinia enterocolitica* bacteremia is associated with iron overload and chelation therapy with deferoxamine. Over the last few years, patient survival has significantly improved because of improved iron chelation therapy, improved modalities to measure liver and cardiac iron load, and a comprehensive care approach. Adherence to chelation is essential for improved clinical outcomes.

Monitoring iron load is key to establishing an individualized, effective iron chelation regimen for each patient. Iron load is determined by serum ferritin, liver iron concentration (LIC), and cardiac iron load. Serum ferritin moderately correlates with body iron stores and is an easy, convenient, and inexpensive measure to trend. However, it

Table 7-1 Complications in TDT and NTDT

Complication	TDT	NTDT	Management	Monitoring
Heart failure	+	+	Iron chelation, standard cardiac care	Cardiac MRI Echocardiogram EKG
Arrhythmia	+	++	Standard care	EKG
Viral hepatitis	+++	+	Hepatitis B vaccination, antiviral therapy	Viral serologies
Hepatic fibrosis, cirrhosis, and hepatocellular carcinoma	++	+++	Standard care	Liver MRI, FibroScan, ultrasound
Growth retardation	++	+	Transfusion, chelation, and hormonal therapy	Clinical growth evaluation
Delayed puberty	++	+	Transfusion, chelation, and hormonal therapy	Tanner stage
Glucose intolerance/diabetes	++	+	Chelation and standard care	Lab monitoring
Decreased bone mineral density	++	+++	Standard and specific therapy	Bone densitometry
Extramedullary masses	+	+++	Hypertransfusions, hydroxyurea, or radiation	Clinical history, CT scan, MRI
Thrombosis	+	+++	Anticoagulation, transfusion	
Pulmonary hypertension	+	+++	Standard care, sildenafil, bosentan	Echocardiogram
Leg ulcers	+	++	Topical treatment, hydroxyurea	

Adapted from Marcon A et al, *Hematol Oncol Clin N Am.* 2018;32:223-236 (© 2018), with permission from Elsevier.

Refer to Cappellini MD et al, eds. Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT). 3rd ed. Thalassaemia International Federation; 2014, and Taher A et al, eds. Guidelines for the Management of Non Transfusion Dependent Thalassaemia (NTDT). Thalassaemia International Federation; 2013, for general guidelines on clinical and laboratory evaluation for complications of thalassemia.

CT, computed tomography; EKG, electrocardiogram.

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has several limitations, including that it is an indirect measure of true body iron burden, is an acute phase reactant, and has a nonlinear response to iron load at high levels. LIC can be determined by liver biopsy or by the new gold standard, liver magnetic resonance imaging (MRI) R2. Normal LIC is <1.8 mg Fe/g dry weight. Cardiac MRI T2★ correlates with cardiac iron load and the risk of developing heart failure increases with T2★ values <20 ms. The risk for developing heart failure is highest when the cardiac T2★ is <8 ms. A complete iron load evaluation includes at least serum ferritin every 3 months, yearly LIC by MRI R2 starting at age 5, and yearly cardiac iron T2★ starting at age 8 to 10. For young children, the risks of sedation should be weighed against the risks of severe liver iron overload.

The main goals of iron chelation therapy are to maintain safe levels of body iron to prevent iron overload and its complications and to reduce accumulated iron. Iron chelation therapy is tailored to each individual based on transfusion rates and iron burden. In TDT, iron chelation therapy with subcutaneous deferoxamine or oral deferasirox is initiated when serum ferritin levels reach approximately 1000 to 1500 ng/mL following approximately 12 months of scheduled transfusions or approximately 20 units of blood. Chelation is adjusted to maintain a ferritin of <1000 ng/mL, an LIC of <5 mg of iron/g dry weight, and a cardiac T2★ of >20 ms. In those with significant cardiac iron burden, combination therapy including deferiprone can reduce cardiac iron. Monitoring for chelator-specific complications should be performed. The different chelators and their properties are summarized in Table 7-2.

Iron overload can occur in NTDT primarily because of increased gastrointestinal absorption in the setting of ineffective erythropoiesis. Therefore, iron overload can occur even in the absence of transfusions. Iron-associated complications are similar to those seen in TDT, except cardiac siderosis is much less common. Serum ferritin and LIC measurements show a moderately positive correlation and should be regularly evaluated in all patients over 10 years old. In NTDT, the total body iron load may be higher than what the serum ferritin levels suggest. Thus a serum ferritin of >800 ng/mL warrants LIC evaluation. Chelation therapy to reduce iron-associated morbidity should be initiated if the LIC is ≥5 mg Fe/g dry weight. Deferasirox has been well studied in NTDT with a good efficacy and safety profile.

Cardiac disease

Cardiovascular complications are the main cause of death in TDT patients. Transfusional cardiac iron overload leads to left ventricular dysfunction and arrhythmias; therefore, echocardiography and electrocardiography (ECG) can be performed annually starting at age 10. Patients with significant myocardial iron can be asymptomatic with normal ventricular function for years before developing heart failure. It is thus important to specifically monitor for cardiac iron load with cardiac MRI T2★. Prevention of cardiac iron overload with chelation adherence must be emphasized. Once cardiac iron overload develops, intensive chelation with monotherapy or combination therapy is key. Cardiac iron overload with heart failure can be successfully reversed with aggressive chelation therapy.

Table 7-2 Properties of iron chelators

	Deferoxamine (DFO)	Deferiprone (DFP)	Deferasirox (DFX)	
			Tablet for oral suspension	Film-coated tablet
Route	SC or IV infusion	Oral (tablet or syrup)	Oral	
Dose	20-60 mg/h over 8-24 h	75-100 mg/kg/d	20-40 mg/kg/d	14-28 mg/kg/d
Schedule	5-7 times a week	3 times daily	Once daily	
Excretion	Urine, feces	Urine	Feces	
Remove liver iron	+++	++	+++	
Remove cardiac iron	++	+++	++	
Side effects	Injection site reaction Allergy High-frequency hearing loss Retinopathy Poor growth *Yersinia* infections	GI (nausea, vomiting, abdominal pain) Increased ALT Arthralgias Neutropenia Agranulocytosis (requires weekly monitoring)	GI (diarrhea, vomiting, nausea, abdominal pain) Skin rash Increased ALT Increased serum creatinine GI bleeding	

ALT, alanine aminotransferase; GI, gastrointestinal, IV, intravenous; SC, subcutaneous.

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Close follow-up with a cardiologist is important because many of these patients also benefit from other cardiac medications.

Pulmonary hypertension is the major cardiovascular complication in NTDT patients. The pathophysiology is multifactorial because of endothelial dysfunction, hypercoagulability, increased vascular tones, inflammation, nitric oxide depletion resulting from hemolysis, and splenectomy. Regularly transfused TDT patients have lower prevalence of pulmonary hypertension, suggesting a therapeutic role for transfusion therapy in NTDT patients who develop this complication. Sildenafil citrate, bosentan, and epoprostenol have been shown to be beneficial in the thalassemia population with pulmonary hypertension.

Liver disease

Liver fibrosis, cirrhosis, and hepatocellular carcinoma are prevalent in patients with b-thalassemia. Patients may benefit from monitoring liver function panels every 3-6 months and consider obtaining liver ultrasound or transient elastography if concern for progressive liver disease. Many adults with TDT have chronic hepatitis C virus (HCV) infection resulting from contaminated RBC products that they received before the early 1990s. The concomitant presence of both HCV infection and iron overload significantly increases the risk of hepatic fibrosis. Treatment with ribavirin-based regimens may be complicated by hemolysis resulting from ribavirin and has been limiting in thalassemia patients. New nonribavirin treatment regimens with direct-acting antivirals have shown sustained viral response rates in thalassemia (97.6%) similar to that in patients without hemoglobinopathies. Thalassemia patients are at risk for hepatocellular carcinoma, especially those with histories of untreated liver iron overload and concurrent HCV infection.

Endocrine complications

Endocrine complications are very common in thalassemia patients, primarily caused by effects of iron deposition in the anterior pituitary or endocrine organs beginning in childhood. Endocrinopathies are generally more common in patients with TDT compared to NTDT because of the significantly increased transfusional iron burden. All TDT and NTDT patients should be routinely followed by an endocrinologist with regular monitoring for endocrinopathies.

Hypogonadotropic hypogonadism (secondary hypogonadism) is the most common endocrinopathy in patients with thalassemia, occurring in 50% to 90% of patients. In children it can present as delayed puberty, whereas in adults decreased libido, infertility, and osteoporosis are common. NTDT patients generally have normal puberty and are fertile due to less iron burden. Appropriate chelation with good adherence is important in preventing hypogonadism.

Diabetes is common in 20% to 30% of adult patients with TDT and strongly correlates with severity of iron overload, inadequate chelation, poor adherence, and late initiation of chelation therapy. Hemoglobin A1c is an unreliable marker of glycemic control in thalassemia patients because of changes in Hb balance and frequent transfusions. Fructosamine is a more reliable marker and can be used to follow diabetic treatment and control, which is similar to the general population. It is indicative of glycemic control over the past 3 weeks.

Hypothyroidism occurs in about 10% of patients with TDT and strongly correlates with the severity of anemia and iron overload. Well-treated patients with TDT are unlikely to develop hypothyroidism. In those with subclinical hypothyroidism, intensification of chelation therapy can help improve thyroid function. Hypoparathyroidism and adrenal insufficiency are less commonly seen in thalassemia patients and are primarily caused by iron overload.

Bone disease

Low bone mass and osteoporosis increase the risk of fracture, and are common in TDT and NTDT patients, occurring in up to 90% of individuals. It tends to be more common in NTDT patients. Contributors to decreased bone mineral density in thalassemia include iron overload with direct iron toxicity on osteoblasts, ineffective erythropoiesis, hypogonadism, iron chelation with deferoxamine, vitamin D deficiency, hypercalciuria, and decreased weight-bearing exercises. Diagnosis involves yearly bone densitometry starting at 10 years old. Treatment involves a multifaceted approach including optimizing transfusions, chelation therapy, treatment of concurrent endocrinopathies, vitamin D replacement, bisphosphonate therapy, physical activity, and smoking cessation. Bisphosphonates have been shown to reduce bone resorption, increase bone mineral density, reduce back pain, and improve quality of life in thalassemia patients.

Other complications

Thalassemia is considered a hypercoagulable state, especially in NTDT where the incidence is as high as 20%. Splenectomy further increases the risk of thrombosis. Overt stroke and silent cerebral infarcts are also increased in thalassemia, especially NTDT. Increased ineffective erythropoiesis in poorly controlled thalassemia results in expansion of extramedullary masses beyond the liver and spleen. Paraspinal masses can cause spinal cord compression. Management involves hypertransfusion, hydroxurea, and in urgent situations, radiation therapy. Leg ulcers are more

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commonly seen in NTDT because of reduced tissue oxygenation and increase with increasing age and iron burden. Additional research is needed to determine the incidence of chronic kidney disease in patients with thalassemia.

Treatment approaches for thalassemia

RBC transfusion

RBC transfusion has been the mainstay in the management of β-thalassemia major and its complications. The goals of transfusions are to promote normal growth and development and to suppress ineffective erythropoiesis. A lifelong chronic blood transfusion program to maintain a pretransfusion Hb level between 9 and 10 g/dL sufficiently suppresses bone marrow expansion while minimizing transfusional iron loading. An increased incidence of cerebral thrombosis, venous thromboembolism, and pulmonary hypertension has been reported in β-thalassemia major and β-thalassemia intermedia following splenectomy, and these risks should be considered before splenectomy. Often, increasing the transfusion targets is sufficient to reduce the degree of splenomegaly.

Luspatercept

Luspatercept is a recombinant fusion protein that binds to transforming growth factor B superfamily ligands, block signaling of SMAD2/3, and enhances erythroid maturation. In the phase 3 multicenter, randomized, double-blind, placebo-controlled BELIEVE trial, adults with b-thalassemia receiving regular transfusion experienced a significant reduction in transfusion burden when receiving subcutaneous dosing of luspatercept as compared to placebo. The primary outcomes was a reduction in transfusion by ≥33% over 12 weeks; 20% of patients were able to reduce their transfusion burden by 1/3. Thromboembolic events were increased in patients receiving luspatercept.

Curative options in thalassemia

Allogeneic bone marrow transplantation from a histocompatible (human leukocyte antigen [HLA]-compatible) sibling has been performed in >1000 thalassemia major patients and is curative in most. The outcome is influenced by the age of the patient and disease status at the time of transplant. The Pesaro prognostic score helps predict transplant outcomes in patients younger than 17 years old. The 3 key prognostic factors, which are indirect estimates of the disease burden and degree of iron overload, include (1) hepatomegaly >2 cm, (2) portal fibrosis, and (3) history of inadequate iron chelation therapy. Over the years, improvements in conditioning regimens, prevention and management of graft-versus-host disease, improved techniques for HLA typing, and overall supportive care have significantly improved overall survival to more than 90% and thalassemia-free survival to more than 80%. Recent studies exploring unrelated donor transplantation, haploidentical transplantation, and nonmyeloablative regimens are encouraging, even in patients with prior iron loading or concomitant HCV infection.

Since allogeneic stem cell transplantation is not available to most patients with thalassemia because of the lack of matched donors, globin gene therapy offers an encouraging new curative approach. Preliminary results in gene therapy for TDT have led to transfusion independence in some subjects and are promising for the future. In addition to gene addition, current gene studies are evaluating the impact of blocking BCL11A. Work continues to determine the optimal factors that influence gene therapy outcomes including patient factors, vector properties, transduction efficiency, and conditioning regimens.

CLINICAL CASE *(continued)*

The patient presented in this case likely has 2 copies of alpha deletions in the trans position (–α/– α) because she is of African descent. Patients with this condition usually have mild microcytic, hypochromic anemia. Targeted RBC forms suggest the presence of thalassemia in an otherwise healthy person. With single or double α-gene deletions, the Hb electrophoresis is typically normal, unlike in β-thalassemia. α-Thalassemia is often a diagnosis of exclusion, and identification of similar findings in family members supports the diagnosis. Molecular testing for specific α-gene deletions confirms the diagnosis. Iron deficiency should be ruled out. Exogenous iron should not be prescribed because it is unnecessary and potentially harmful. Patients are generally asymptomatic, require no treatment, and have a normal life expectancy.

KEY POINTS

- The thalassemias are characterized by a reduced rate of synthesis of one of the globin subunits of the Hb molecule.
- The intracellular precipitation of the excess, unpaired globin chains in thalassemia damages red cell precursors and circulating red cells, resulting in ineffective erythropoiesis and hemolysis.
- The α-thalassemias are primarily caused by DNA deletions. Four α-genes are normally present, so multiple phenotypes are possible when gene deletions occur.
- The β-thalassemias are caused by >200 different mutations, usually point mutations, with a wide variety of genetic abnormalities documented.

Key Points continue

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KEY POINTS *(continued)*

- α-Thalassemia trait is characterized by mild asymptomatic anemia with microcytic indices and a normal Hb electrophoresis.
- The Hb electrophoresis in β-thalassemia trait reveals increased levels of HbA_2 and variably increased HbF.
- Thalassemia can be clinically classified into transfusion-dependent thalassemia (TDT) or non-transfusion-dependent thalassemia (NTDT).
- Patients with TDT require regular blood transfusions for survival, whereas those with NTDT who have a mild-to-moderate phenotype require intermittent transfusions during periods of acute illness, infection, or pregnancy, or to allow for normal growth and development.Iron overload is a complication of TDT and NTDT, and monitoring of iron load with serum ferritin, and liver and cardiac iron content by MRI are important to optimize chelation therapy initiation and management.
- Hemolytic anemia, ineffective erythropoiesis, and iron overload contribute to multiple complications of TDT and NTDT including bone deformities, cardiac failure, arrhythmia, liver cirrhosis, HCV infection, thrombosis, endocrinopathies, osteoporosis, leg ulcers, and pulmonary hypertension.
- Partner testing and genetic counseling in individuals with α-thalassemia trait is important so that a pregnant woman with a risk of a homozygous α^0-thalassemia fetus can consider further testing, early termination, or undergo intrauterine transfusions to support fetal growth should they wish to maintain the pregnancy.

Sickle cell disease

CLINICAL CASE

A 17-year-old African American male with homozygous sickle cell anemia (HbSS) is admitted to the hospital with a 4-day history of a typical painful episode involving his arms and legs. There is no recent febrile illness. Past medical history is remarkable for few hospital admissions for pain crises and red blood cell transfusion once as a young child. He is in severe pain and appears ill, and vital signs are remarkable for a pulse of 129 and temperature of 38.5°C. Scleral icterus and moderate jaundice are noted. Laboratory data include hemoglobin 7.2 g/dL (baseline 9.1 g/dL), reticulocyte count of 2%, absolute reticulocyte count of 0.132×10^6/L, and platelet count 72,000/μL. Liver function tests are elevated above baseline and include a direct bilirubin of 4.8 mg/dL, aspartate aminotransferase of 1200 U/L, and alanine aminotransferase 1550 U/L. His creatinine is elevated at 4.3 mg/dL. Abdominal ultrasound is nondiagnostic. He is immediately started on intravenous fluids and opioid analgesics. Broad-spectrum antibiotics are empirically administered. Over the next 24 h he becomes tachypneic and slightly confused. Hypoxemia develops despite oxygen supplementation, and anuria ensues. Serum creatinine has increased to 6.4 mg/dL, direct bilirubin to 7.8 mg/dL, aspartate aminotransferase to 2725 U/L, and creatine phosphokinase to 2200 IU/L and Hb has decreased to 5.8 g/dL. The patient undergoes simple transfusion and subsequently red blood cell exchange. Acute dialysis is required. He slowly improves during a prolonged 3-week hospitalization. No infectious etiology was identified.

Sickle hemoglobin (HbS) was the first Hb variant discovered. It has been well characterized at the biochemical and molecular level. Heterozygosity for the sickle cell gene (β^S), called *sickle cell trait,* occurs in >20% of individuals in equatorial Africa; up to 20% of individuals in the eastern provinces of Saudi Arabia and central India; up to 6.3% in Hispanic populations; and approximately 5% of individuals in parts of the Mediterranean region, the Middle East, and North Africa. In HbS, a hydrophobic valine is substituted for the normal, more hydrophilic glutamic acid at the sixth residue of the β-globin chain. This substitution is caused by a single nucleotide mutation (GAG/GTG) in the sixth codon of the β-globin gene. Heterozygous inheritance of HbS offers a degree of protection from severe malaria infection. This has been offered as an explanation for the evolutionary selection of the HbS gene despite the devastating effects of the homozygous state. The β^S gene is inherited in an autosomal codominant fashion. That is, heterozygous inheritance does not cause disease, but is detectable (sickle cell trait); homozygous inheritance or compound heterozygous inheritance with another mutant β-globin gene results in disease. The *sickle cell syndromes* include all conditions with E6V mutation, mostly when β^S is inherited (including sickle cell trait). In contrast, the term *sickle cell disease* includes only those genotypes associated with varying degrees of chronic hemolytic anemia and vaso-occlusive pain (not sickle trait): homozygous sickle cell anemia (HbSS), sickle-HbC disease (HbSC), sickle β^0-thalassemia ($HbS\beta^0$), and sickle β^+-thalassemia ($HbS\beta^+$). Less common Hb mutants, such as O^{Arab}, D^{Punjab}, or E, may be inherited in compound heterozygosity with β^S to result in sickle cell disease. Finally, the term *sickle cell anemia* includes the more severe forms of sickle cell anemia including homozygous sickle cell anemia (HbSS), and sickle β^0-thalassemia ($HbS\beta^0$).

Sickle cell trait

Sickle cell trait (HbAS) occurs in 8% to 9% of the African American population. The vast majority of individuals with sickle cell trait do not have any clinical manifestations. However, sickle cell trait is a risk factor for the rare

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complications of hematuria, renal papillary necrosis, pyelonephritis during pregnancy, and risk of splenic infarction at high altitude. Sickle trait also is associated with the extremely rare medullary carcinoma of the kidney, and an increased risk of sudden death during extreme conditions of dehydration and hyperthermia. Recent publications have shown that individuals with sickle trait are at higher risk of chronic kidney disease and venous thromboembolism. The risk for kidney disease is likely modified by other underlying comorbidities, and the risk of venous thromboembolism is low, similar to other low-risk thrombophilias such as factor V Leiden and prothrombin gene mutation. Individuals with sickle cell trait generally have a HbA:HbS ratio of approximately 60:40 because of the greater electrostatic attraction of α chains to β^A rather than β^S chains. When the availability of α chains is limited by coinheritance of 1 or more α-thalassemia deletions, the HbA:HbS ratio is further increased.

Pathophysiology

The hallmark of sickle cell pathophysiology is the intraerythrocytic polymerization of deoxyhemoglobin S. When deoxygenation of HbS occurs, the normal conformational change of the tetramer exposes on its external surface a hydrophobic β_6 valine (instead of the hydrophilic glutamate of HbA), resulting in decreased solubility and a tendency of deoxyhemoglobin S tetramers to aggregate or polymerize. The rate and degree of this polymerization determines the rheologic impairment of sickle erythrocytes and the change in morphology for which the condition was named. Polymerization rate and extent are related to the intracellular concentration of HbS, the type and fractional content of other Hbs present (particularly HbF), and percent oxygen saturation. These variables correlate with the rate of hemolysis in sickle cell syndromes.

Multiple factors determine the clinical manifestations of sickle cell disease. In addition to physiologic changes such as tissue oxygenation and pH, multiple genetic polymorphisms and mutations may modify the presentation of the disease. This is best appreciated by examining the influence of the coinheritance of other Hb abnormalities on the effects of HbS. For example, the coexistence of α-thalassemia reduces the hemolytic severity as well as the risk of cerebrovascular accidents. High levels of HbF may substantially reduce symptoms as well as clinical consequences. Compound heterozygosity for a second abnormal Hb (eg, HbC, HbD, or HbE) or β-thalassemia also modifies some of the manifestations of disease (discussed later in this section) (Table 7-2).

Several restriction fragment-length polymorphisms (RFLPs) may be identified in the vicinity of a known gene and define the genetic background upon which a disease-causing mutation has arisen. For example, the coinheritance of a defined set of RFLPs around the β-globin gene can define a disease-associated "haplotype" that marks the sickle mutation within a specific population. These β-globin haplotypes have also been associated with variations in disease severity. This association is probably not related to the RFLPs themselves, but rather is mediated through linked differences in γ-chain (HbF) production. The β^S gene has been found to be associated with 5 distinct haplotypes, referred to as the Benin (Ben), Senegal (Sen), Central African Republic (CAR or Bantu), Cameroon (Cam), and Arab-Indian (Asian) haplotypes. This is evidence that the β^S gene arose by 5 separate mutational events. In general, the Asian and Sen haplotypes may be associated in a delay in clinical manifestations earlier in life because of fetal Hb expression, and CAR is associated with a more severe early clinical course.

Although the deoxygenation-polymerization-sickling axiom provides a basic understanding of sickle cell disease, there is an increasing appreciation that interactions of sickle cells with other cells and proteins contribute to the hemolytic and vaso-occlusive processes. The chronic hemolytic nature of sickle cell disease leads to chronic depletion of nitric oxide both from the release of arginase and also free heme. Free heme is associated with impaired cleavage of large von Willebrand factor multimers by ADAMTS 13 and also with the activation of toll-like receptor 4 (TLR4). Heme-induced TLR4 has been shown to cause both endothelial activation and vaso-occlusion in murine models of sickle cell disease. In vitro data show that sickle erythrocytes exhibit abnormally increased adherence to vascular endothelial cells, as well as to subendothelial extracellular matrix proteins. Apparent endothelial damage is demonstrated by the increased number of circulating endothelial cells in sickle cell disease patients, and by the increase in such cells during vaso-occlusive crises. Activation of blood coagulation, resulting in enhanced thrombin generation and evidence for platelet hyperreactivity, has been demonstrated in patients with sickle cell disease during steady-state and vaso-occlusive episodes. It has been suggested that the exposure of RBC membrane phosphatidylserine and circulating activated endothelial cells in sickle cell disease patients contribute to the hypercoagulability by providing procoagulant surfaces. The correlation of elevated white blood cell counts to increased mortality and adverse outcomes identified by epidemiologic studies of sickle cell disease patients suggest that neutrophils also participate in vaso-occlusion. This concept has been further supported by the precipitation of vaso-occlusive episodes with markedly increased neutrophil counts associated with the administration of

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granulocyte colony-stimulating factor. These findings taken together support the concept that the products of multiple genes, as well as inflammatory cytokines, contribute to the pathology of sickle cell disease.

Laboratory features

The diagnosis of the sickle cell syndromes is made by the identification of HbS in erythrocyte hemolysates. Historically, cellulose acetate electrophoresis at alkaline pH was used to separate HbA, HbA_2, and HbS, and citrate agar electrophoresis at acidic pH was used to separate comigrating HbD and HbC from HbS and HbA_2, respectively. Currently, high-performance liquid chromatography (HPLC) and isoelectric focusing are used in most diagnostic laboratories to separate Hbs. In both HbSS and $S\beta^0$-thalassemia, no HbA is present. HbA_2 is elevated in $S\beta^0$-thalassemia, but it also can be nonspecifically elevated in the presence of HbS, so an elevation of A_2 alone cannot reliably distinguish HbSS from $S\beta^0$-thalassemia Evaluating the MCV can help differentiate HbSS from $HbS\beta^0$-thalassemia. In HbSS, the MCV is normal, whereas in $HbS\beta^0$-thalassemia, the MCV is reduced. Review of the peripheral smear reveals the presence of irreversibly sickled cells in both HbSS and $HbS\beta^0$-thalassemia (Figure 7-7), but only rarely in $HbS\beta^+$-thalassemia and HbSC. In sickle cell trait and $S\beta^+$-thalassemia, both HbS and HbA are identified but with different ratios. The HbA:HbS ratio is 60:40 in sickle trait (more HbA than HbS) and the HbA:HbS ratio is approximately 15:85 in $S\beta^+$-thalassemia (more HbS than HbA). Microcytosis, target cells, and anemia can additionally differentiate $HbS\beta^+$-thalassemia from sickle trait (Table 7-2). Turbidity tests (for HbS) are positive in all sickle cell syndromes, including HbAS (sickle trait). The classic sickle cell slide test or "sickle cell prep" (using sodium metabisulfite or dithionite) and the turbidity test detect only the presence of HbS, so they do not differentiate sickle cell disease from sickle cell trait. Therefore, they have limited utility. Sickle cell disease can be diagnosed by DNA testing of the preimplanted zygote in the first trimester of pregnancy using chorionic villus sampling, in the second trimester using amniocentesis, or after birth using peripheral blood.

Clinical manifestations

The clinical manifestations in sickle cell disease are associated with the pathophysiology of shortened RBC survival (hemolytic complications and exposure to free heme/Hb), endothelial dysfunction associated with vaso-occlusion as well as hyperviscosity in patients with milder sickle cell genotypes. The erythrocyte lifespan is shortened from the normal 120 days to approximately 10 to 25 days, resulting in moderate to severe hemolytic anemia, with a steady-state mean Hb concentration of 8 g/dL (ranging from 6 to 9 g/dL) in HbSS disease. The anemia is generally well tolerated because of compensatory cardiovascular changes and increased levels of 2,3-BPG. Although patients have chronic anemia, several conditions can exacerbate this anemia and lead to acute symptomatic anemic events. (Table 7-3). The transient aplastic crisis resulting from erythroid aplasia is often caused by human parvovirus infection as well as other infections, which may result in severe or life-threatening anemia. Lesser degrees of bone marrow "suppression" are associated with other infections. Sudden anemia may be caused by acute splenic sequestration in children with HbSS or $S\beta^0$ (and in adults with HbSC or $S\beta^+$-thalassemia) or, less frequently, hepatic sequestration, concomitant glucose-6-phosphate dehydrogenase (G6PD) deficiency, or superimposed autoimmune hemolysis. Chronic exacerbations of anemia may be the result of folate or iron deficiency or reduced erythropoietin levels caused by chronic renal insufficiency. Because of the chronic erythrocyte destruction, patients with sickle cell disease have a high incidence of pigmented gallstones, which are often asymptomatic.

There are many important clinical differences among the genotypes that cause sickle cell disease (Table 7-2). HbSS is associated with the most severe anemia, most frequent pain, and shortest life expectancy (median age of 54 years for men and women in most recent study), although there is tremendous heterogeneity in these variables even

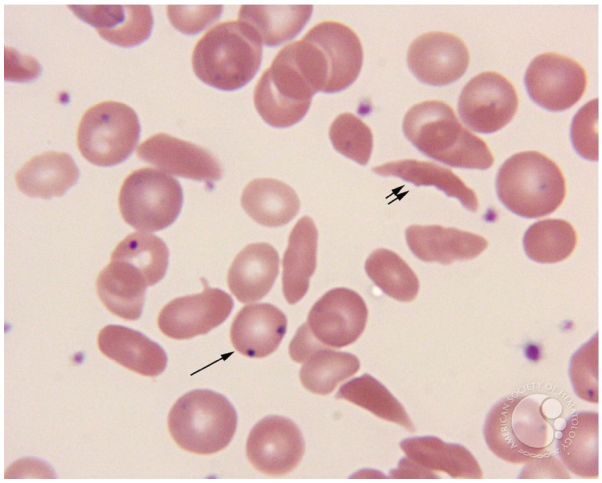

Figure 7-7 Irreversibly sickled cell. This peripheral blood film shows an irreversibly sickled cell (ISC) that occurs in sickle cell anemia (SS), Sb^0-thalassemia (double arrow). ISCs are rare in hemoglobin SC and Sb^+-thalassemia. Also note the Howell-Jolly bodies in this view (single arrow). Source: ASH Image Bank/John Lazarchick (image 00003961).

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Table 7-3 Typical clinical and laboratory findings of the common forms of sickle cell disease after 5 years old

Disease	Clinical severity	S, %	F, %	A_2, %*	A, %	Hemoglobin, g/dL	MCV, fL
SS	Usually marked	>90	<10	<3.5	0	6-9	>80
Sb^0	Marked to moderate	>80	<20	>3.5	0	6-9	<70
Sb^+	Mild to moderate	>60	<20	>3.5	10-30	9-12	<75
SC	Mild to moderate	50	<5	0†	0	10-15	75-85
S^- HPFH	Asymptomatic	<70	>30	<2.5	0	12-14	<80

HPFH, hereditary persistence of fetal hemoglobin.

*HbA_2 can be increased in the presence of HbS, even in the absence of β-thalassemia. The classical findings are shown here.

†Fifty percent of hemoglobin C migrates near hemoglobin A_2 on alkaline gel electrophoresis or isoelectric focusing.

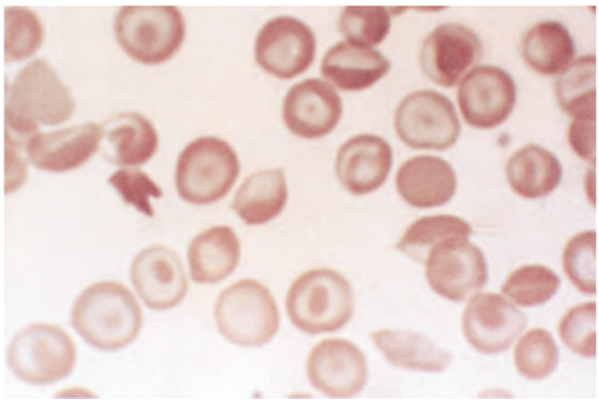
within this genotype. $HbS\beta^0$-thalassemia can closely mimic HbSS, despite the smaller red blood cells, lower MCH concentrations, and higher levels of HbF and HbA_2 associated with this genotype. Patients with HbSC generally live longer lives (median age of 60 years for men and 68 years for women) and have less severe anemia (~20% are not anemic at all), higher MCH concentrations, and less frequent pain, but they have more frequent ocular and bone complications. Although HbC does not enter into the deoxyhemoglobin S polymer, patients with HbSC have symptoms, whereas those with sickle cell trait (HbAS) do not. This is thought to be caused by 2 important consequences of the presence of HbC: the HbS content in HbSC is 10% to 15% higher than that seen in sickle cell trait (HbS of approximately 50% versus 40%), and the absolute intraerythrocytic concentration of total Hb is increased. The latter phenomenon results from persistent loss of cellular K^+ and water from these cells induced by the toxic effect of HbC on cell membranes. Another effect of this dramatic cellular dehydration is the generation of target cells, which are far more prevalent on the peripheral smear than sickled forms (Figure 7-8). Finally, in HbSC disease, the increased hematocrit combined with the higher MCH concentration (MCHC) and cellular dehydration results in higher whole blood viscosity, which may increase the likelihood of vaso-occlusion. Patients with $HbS\beta^+$-thalassemia have less severe anemia and pain than patients with $HbS\beta^0$-thalassemia. This is the result of smaller cells, lower MCHC, increased content of HbF and HbA_2 and, most importantly, the presence of significant amounts (10% to 30%) of HbA that interferes with polymerization.

Sickle cell disease is a multisystem disorder. Organ systems subject to recurrent ischemia, infarction, and chronic dysfunction include the lungs (acute chest syndrome, pulmonary fibrosis, pulmonary hypertension, hypoxemia), central nervous system (overt and covert cerebral infarction, subarachnoid and intracerebral hemorrhage, seizures, cognitive impairment, moyamoya disease, cerebral vasculopathy), cardiovascular system (cardiomegaly, congestive heart failure), genitourinary system (hyposthenuria, hematuria, proteinuria, papillary necrosis, glomerulonephritis, priapism), spleen (splenomegaly, splenic sequestration, splenic infarction and involution, hyposplenism), eyes (retinal artery occlusion, proliferative sickle retinopathy, vitreous hemorrhage, retinal detachment), and skin (leg ulcerations). The risk of life-threatening septicemia and meningitis because of encapsulated organisms, such as *Streptococcus pneumoniae,* is markedly increased in children with sickle cell disease. This susceptibility is related to functional and anatomic asplenia and decreased opsonization because of deficient production of natural antibodies. The risk for such infections persists into adulthood.

Painful episodes

The acute, painful vaso-occlusive crisis is the stereotypical and most common complication of sickle cell disease. These often-unpredictable events are thought to be caused by obstruction of the microcirculation by erythrocytes and other blood cells, leading to painful tissue hypoxia and infarction. They most commonly affect the long bones,

Figure 7-8 Sickle-hemoglobin C disease. This peripheral blood film shows no irreversibly sickled cells, as expected for hemoglobin SC, but shows instead a large number of target cells and several dense, contracted, and folded cells containing aggregated and polymerized hemoglobin.



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back, chest, and abdomen. Acute pain events may be precipitated by dehydration, cold temperatures, exercise (particularly swimming), pregnancy, infection, or stress. Often no precipitating factor can be identified. Painful episodes may or may not be accompanied by low-grade fever. Of concern, it has been estimated that 30% to 40% of adolescent and adult patients with sickle cell anemia will progress from acute intermittent pain events to chronic pain.

Painful episodes can be associated with serious complications, and a high frequency of pain is a poor prognostic factor for survival. Acute pain unresponsive to rest, hydration, and oral analgesics at home requires prompt attention and is the leading cause of hospitalization. It is essential to consider infectious and other etiologies of pain in the febrile patient. A complete blood count should be obtained. Because some degree of negative fluid balance often is present, oral or intravenous hydration is important. Caution must be used with intravenous hydration of adults especially, who may have decreased cardiac reserve. It is important to provide rapid administration of analgesics and the selection of agents should be individualized based on previous experience. Parenteral opioids, preferably morphine or hydromorphone, are often necessary for both children and adults. The addition of nonsteroidal anti-inflammatory drugs, such as ibuprofen or ketorolac, may decrease the requirement for opioid analgesics but should be used with appropriate vigilance in sickle cell disease because of potential nephrotoxicity. Maintenance analgesia can be achieved with patient-controlled analgesia pumps or with administration at fixed intervals. Constant infusion of opioids requires close monitoring because the hypoxia or acidosis resulting from respiratory suppression is particularly dangerous. Meperidine is discouraged because of its short half-life and the accumulation of the toxic metabolite normeperidine, which lowers the seizure threshold. Use of pain assessment instruments and attention to the level of sedation at regular intervals are necessary. Oxygen supplementation is not required unless hypoxemia is present. The use of incentive spirometry has been shown to reduce pulmonary complications in patients presenting with chest or back pain. It has been demonstrated that the number of hospitalizations for painful events can be reduced by prompt intervention in an outpatient setting dedicated to sickle cell disease management. Nonpharmacologic management techniques should be considered, as well as evaluation for depression for the patient with frequent episodes or chronic pain. Blood transfusion is not indicated in the treatment of uncomplicated painful episodes.

Acute chest syndrome

The diagnosis of acute chest syndrome is based on a new radiographic pulmonary infiltrate on chest x-ray associated with symptoms such as fever, cough, and chest pain. It is often difficult to distinguish acute chest syndrome from infectious pneumonia. As the nonspecific term implies, various insults or triggers can lead to acute chest syndrome and treatment for infectious pneumonia should be concurrent. In children, fever is a common presenting symptom, whereas chest pain is more common in adults. Acute chest syndrome often develops in patients who initially present only with an acute painful event. Young age, low HbF, high steady-state Hb, and elevated white blood cell count in steady state have been identified as risk factors. In a multicenter prospective study, bacterial (often atypical) or viral infections accounted for approximately 30% of episodes, whereas fat emboli from the bone marrow were responsible for approximately 10% of events, with pulmonary infarction as another common suspected cause. Early recognition of the condition is of utmost importance because acute chest syndrome has become

Table 7-4 Causes of acute exacerbations of anemia in sickle cell disease

Cause	Comment
Aplastic crisis	Caused by human parvovirus; does not recur
Acute splenic sequestration crisis	Often recurrent in childhood before splenic involution
Acute chest syndrome	Anemia may precede the onset of respiratory signs and symptoms
Vaso-occlusive crisis	Minimal decline only
Hypoplastic crisis	Mild decline; accompanies many infections
Accelerated hemolysis	Infrequent; accompanies infection of concomitant G6PD deficiency
Delayed hemolytic transfusion reaction	Acute drop in hemoglobin after transfusion, possibly to below pretransfusion Hb caused by bystander effect
Hepatic sequestration	Rare
Folate deficiency (megaloblastic crisis)	Rare, even in the absence of folate supplementation

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the leading cause of death for both adults and children with sickle cell disease.

Management includes maintaining adequate oxygenation and administration of antibiotics to address the major pulmonary pathogens and community-acquired atypical organisms. Fluid management needs particular attention to prevent pulmonary edema by limiting oral and intravenous hydration to 1.0 to 1.5 times maintenance (after correction of any dehydration). Pain control to avoid excessive chest splinting and the use of incentive spirometry are key adjunctive measures. Bronchodilator therapy is effective if there is associated reactive airway disease, which is particularly common in children. Transfusion of RBCs should be considered if there is hypoxemia or acute symptomatic exacerbation of anemia. Exchange transfusion should be performed for hypoxemia despite oxygen supplementation, widespread (bilateral, multilobar) infiltrates, and rapid clinical deterioration. Patients with acute chest syndrome are at risk for recurrences as well as subsequent chronic lung disease. Preventive measures include hydroxyurea therapy and chronic RBC transfusions.

Central nervous system disease

Without primary prevention, overt stroke may occur in 11% of young sickle cell anemia patients (but is much less common in SC disease and $S\beta^+$-thalassemia), accounting for significant morbidity and mortality. The majority of overt strokes result from ischemic events involving large arteries with associated vascular endothelial damage, including intimal and medial proliferation. Hemorrhagic events are more common in adults and may result from rupture of collateral vessels (moyamoya) near the site of previous infarction. Suspicion of a neurologic event requires emergent imaging with computed tomography to assess for hemorrhage, followed by MRI. The more frequent use of neuroimaging has identified a substantial incidence of subclinical cerebrovascular disease including silent cerebral infarcts and cerebral vasculopathy.

An overt stroke is an emergency. The acute management of overt stroke includes transfusion, usually by an exchange technique, to reduce the HbS percentage to <30%. For patients that have suffered an overt stroke, chronic transfusion therapy to maintain the HbS <30% as the pretransfusion target decreases the chance of recurrent overt stroke but does not eliminate it. After 3 to 5 years of such transfusions and no recurrent neurologic events, some physicians "liberalize" the transfusion regimen to maintain the HbS <50%. The optimal duration of transfusions is not known, and they often are continued indefinitely. A pediatric randomized controlled trial (the Stroke with Transfusions Changing to Hydroxyurea [SWiTCH] study) of continued chronic transfusions versus hydroxyurea for long-term secondary stroke prevention was stopped early because of futility, and there was an excess of recurrent strokes in the hydroxyurea arm ($N = 7$) compared with continued transfusions ($N = 0$).

Abnormally increased blood flow velocity on a stroke screen (transcranial Doppler [TCD]) can identify children with HbSS at high risk of primary overt stroke. A randomized controlled trial of prophylactic transfusions versus observation for children with abnormal TCD velocities showed a reduced risk of the first stroke in patients receiving transfusions (the Stroke Prevention Trial in Sickle Cell Anemia [STOP] study). The results of a phase 3, primary stroke prevention, multicenter, randomized controlled trial for children with abnormal TCD velocities and normal cerebral vasculature (the TCD With Transfusions Changing to Hydroxyurea [TWiTCH] study) showed that for patients placed on blood transfusion therapy for elevated TCD velocities for at least 12 months, switching to maximum tolerated doses of hydroxyurea was noninferior to continued chronic blood transfusion therapy.

Silent cerebral infarcts are one of the most common neurologic complications of sickle cell anemia, occurring in about 30% of adolescents and 50% of adults. A randomized clinical trial assigned children of ages 5 to 15 years with sickle cell anemia to receive regular blood transfusions or observations. Regular blood transfusion therapy significantly reduced the incidence of new central nervous system (CNS) events (6% in treatment arm versus 14% in observation arm). Limited pediatric data suggest a benefit of hydroxyurea for prevention or treatment of prior silent cerebral infarcts. Additional long-term studies are needed to understand the natural history of silent cerebral infarcts and interventions to prevent progression.

No randomized controlled trials have evaluated the benefit for revascularization surgery in patients with moyamoya syndrome or stroke. In addition, there is currently no evidence to guide management of sickle cell patients with cerebrovascular accidents and neurologic events, but in general, the approach is extrapolated from the trials in patients with SS and $S\beta^0$ disease. There are also current evaluation guidelines for the evaluation of transient ischemic attacks in the American Society of Hematology Guidelines.

Pulmonary disease

While the primary mechanism of vaso-occlusion in SCD is polymerization of sickle Hb in the vasculature, changes in the red blood cell membrane and function, and in the endothelium also play a significant role in its pathophysiology. Because of these alterations, patients with SCD frequently

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present with both acute as well as chronic pathology in the pulmonary parenchyma and vasculature. Indeed, acute pulmonary complications of SCD have been noted in the literature dating back to the 1930s, when Steinberg described autopsy results of a sickle cell patient that contained infarcts as well as thrombi in the lung. Even as childhood mortality rates in SCD improve, pulmonary diseases account for an increasing amount of morbidity and mortality in an aging SCD population. Acute complications include asthma exacerbation, acute chest syndrome, and thromboembolic disease, while chronic complications include sickle cell lung disease, asthma, obstructive sleep apnea, and pulmonary hypertension.

Pulmonary hypertension

Because of the chronic hemolysis associated with SCD, as well as repeated injury to the lung vasculature, patients with SCD have an increased risk of pulmonary hypertension. Although right heart catheterization is considered the gold standard for diagnosis of pulmonary hypertension, given the invasive nature of the test, transthoracic Doppler echocardiography (TTE) is frequently used as a means of screening.

While echo screening shows elevated pressures in 20% to 60% of the sickle cell patient population, a prospective study from France demonstrated an elevated Tricuspid regurgitant jet velocity (TRJV) in 27% of 398 patients. However, follow-up right heart catheterization in all patients with TRJV more than 2.5 m/s revealed elevated mean pulmonary arterial pressure over 25 mm Hg in only 6% of the patient population, leading the researchers to conclude that TTE may overestimate the prevalence of PHT. Subsequent studies have shown right heart–confirmed PHT is present in ~10% of patients. And, while patients with elevated TRJV did not all have pulmonary hypertension, in the National Institutes of Health (NIH) study, of the 62 patients with elevated TRJV jet of at least 2.5 m/s and a median follow-up time of 17.3 months, the rate ratio for death was 10:1, indicating a significant increase in mortality.

Several screening guidelines differ in recommendations as to the screening for PHT in sickle cell disease. The 2014 NIH guidelines and more recent ASH guidelines do not recommend screening, except in symptomatic patients as outlined in Table 7-5.

In adults and children with TRJV >2.5 m/s with symptoms, right heart catheterization is recommended for confirmation of the diagnosis. Of note, screening (as well as diagnostic) TTE should be done in a steady state as acute crisis can increase pulmonary pressures. Treatment of PHT requires both treatment of the underlying sickle cell disease as well as PHT-specific therapy managed by a PHT expert.

Cardiac disease

Cardiovascular disease remains a significant cause of morbidity and mortality in sickle cell disease. Prior data from studies involving autopsy series have shown that presence of myocardial infarcts in absence of obstructive epicardial vessel disease. SCD patients also have impaired myocardial perfusion reserve at steady state as compared to healthy controls. Patients with sickle cell disease are also at increased risk of ventricular tachycardia and sudden cardiac death. Although the conventional risk factors such as hyperlipidemia and hypertension do seem to play a primary role in the pathophysiology, MRI imaging shows areas of microvascular disease as well as scaring. Additionally, recent studies show elevation of the specific inflammatory marker interleukin-18 in animal models as well as patients at risk of sudden cardiac death. While iron overload is less common in sickle cell disease compared to b-thalassemia, increased cardiac iron overload can lead to significant morbidity and mortality.

Renal disease

Patients with sickle cell disease are at an increased risk for developing kidney disease. The renal system requires a hypertonic, hyperosmotic environment, in addition

Table 7-5 Symptoms that prompt further screening for pulmonary hypertension (PH) in sickle cell disease (SCD)

Dyspnea at rest or with exertion that is out of proportion to known condition, increased compared with baseline, or unexplained
Hypoxemia at rest or with exertion that is out of proportion to known condition, increased compared with baseline, or unexplained
Chest pain at rest or with exertion that is out of proportion to known condition, increased compared with baseline, or unexplained
Increase in exercise limitation compared with baseline that is unexplained by other factors
History of recurrent hypoxemia at rest or with exertion
Evidence for sleep-disordered breathing with or without hypoxemia
History of syncope or presyncope
Evidence for loud P2 component of second heart sound or unexpected or new murmur on examination
Signs of heart failure and/or fluid overload on examination
History of pulmonary embolism
Patients with SCD who also have comorbid conditions (eg, connective tissue disease)
Patients with SCD who have disease complications (eg, leg ulcers, priapism) known to be associated with PH when signs or symptoms of PH are present

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to relative hypoxia, for the countercurrent exchange to occur. This environment likely accounts for the high prevalence of urine concentrating defects, renal papillary necrosis, and tubular defects seen in sickle cell patients. In addition, glomerulopathy occurs leading to progression to chronic kidney disease and the development of end-stage renal disease. Patients often experience hyperfiltration early in life and develop albuminuria as early as the first decade. Over time, the glomerular filtration rate declines and albuminuria increases. It is vital to monitor progression and prevent the development of end-stage renal disease as 1/4 of patients die within the first year of starting dialysis and sickle cell patients still experience difficulties receiving a kidney transplant. All sickle cell patients should be monitored annually starting at age 10 for the development of albuminuria; if present, especially >100 mg/g, clinicians should consider initiating renoprotective therapies.

Pregnancy and reproductive health

All patients with sickle cell disease should receive counseling about their reproductive health and family planning. Men and women of reproductive age should receive genetic counseling; partners of people with SCD that do not know their SCD or thalassemia status should be referred for hemoglobinopathy screening. Pregnancy poses some risk to the mother as well as to the fetus. Spontaneous abortions occur in approximately 5% of pregnancies in sickle cell anemia, and preeclampsia occurs at an increased frequency in sickle cell disease. Low birth weight, preterm labor, and premature delivery are common. All patients should be followed in a high-risk prenatal clinic, ideally at 2-week intervals with close consultation with a hematologist. Patients should receive folic acid 1 mg/d, in addition to the usual prenatal vitamins and should be counseled regarding the additional risks imposed by poor diet, smoking, alcohol, and substance abuse. Data do not support the routine use of prophylactic transfusions. Simple or exchange transfusions, however, should be instituted for the indications outlined previously, as well as for pregnancy-related complications (eg, preeclampsia). Close follow-up is indicated postpartum when the patient is still at high risk for complications. The option of contraception with an intrauterine device, subcutaneous implant, progesterone-only contraceptives, or condoms should be discussed with all women of childbearing age.

Bone complications

One of the first manifestations of sickle cell disease, acute dactylitis (hand-foot syndrome), results from bone marrow necrosis of the hands and feet. The first attack generally occurs between 6 and 18 months of life, when the HbF level declines. Dactylitis is uncommon after age 3 years, as the site of hematopoiesis shifts from the peripheral to the axial skeleton. Long-bone infarcts with pain and swelling may mimic osteomyelitis. Other skeletal complications of sickle cell disease include osteomyelitis, particularly because of *Salmonella* and staphylococci, and avascular necrosis, especially of the femoral and humeral heads. In addition to these acute complications, many patients experience chronic bone complications including avascular necrosis (often of the femoral or humeral head) and bone mineral density complications.

Ophthalmologic complications

Patients with sickle cell may suffer from various ophthalmologic complications. One classic conjunctival abnormality in sickle cell patients is that of "comma-shaped" vessels. Ophthalmic screening is important to identify other changes that typically involve the retina. Nonproliferative sickle cell retinopathy may present with occlusion of peripheral retinal vasculature described as "salmon patches," which represent retinal hemorrhage from superficial blood vessels. Over time, resorption of blood may result in retractile deposits in the retina and secondary retinal pigment epithelium changes that result in black sunburst spots. Reduction in vision is typically secondary to occlusion of the perifoveal capillaries, which can be detected as thinning of the retina using Optical Coherence Tomography imaging. Vaso-occlusion of the retina and choroid can lead to ischemic changes that may result in vascular occlusion, arteriovenous anastomoses, and neovascularization, often described as a "sea fan." These proliferative changes can further lead to acute vision loss from vitreous hemorrhage and ultimately traction and or rhegmatogenous retinal detachment. There are also nonproliferative changes that can lead to vision loss. Specifically, the areas of hemorrhage and decreased blood flow can lead to salmon patch hemorrhages, black sunburst lesions, and sickle cell maculopathy and thinning of the retina. Additionally, acute occlusion of the retinal vessel can lead to sudden, painless vision loss and is considered an ischemic stroke equivalent. This is frequently similar to an acute stroke, with urgent need for exchange transfusion as well as ocular decompression to maximize blood flow.

Additional complications

Liver disease

Because of chronic hemolysis, sickle cell patients have a high incidence of gallstones. Ultrasound can be performed to identify cholelithiasis and clinicians can

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consider referral for cholecystectomy in patients with acute complications or chronic painful events from gallstones. Additionally, sickle cell can lead to several different types of acute and chronic complications. Acute sickling of red blood cells in the liver can lead to an acute sickle hepatic crisis or hepatic sequestration. Patients will present with right upper-quadrant pain and hepatomegaly with transaminitis and elevated conjugated bilirubin. Chronic liver disease and cirrhosis can occur with recurrent hepatic ischemic insults.

Priapism

Priapism is a frequent genitourinary (GU) complication in males. Evidence-based guidelines are lacking for appropriate management to prevent or treat events; however, events lasting >1 hour require emergent urologic interventions. The 2 main forms of priapism are ischemic and stuttering priapism. Ischemic priapism is the most common form and is caused by reduced or absent blood flow. Stuttering priapism refers to episodes that start and end in a shorter time frame but with greater frequency.

Leg ulcers

Sickle cell leg ulcers are often located in the lower extremities, specifically where there is poor circulation, thin skin, and less subcutaneous fat (eg, perimalleolar). Ulcers can be singular, stuttering, or chronic, and frequently recur at the site of previous ulcerations. Risk factors include prior trauma, HbSS genotype, male sex, increasing age, and reduced HbF levels. There is no statistical difference in prevalence of leg ulcers among hydroxyurea and nonhydroxyurea patients. Treatments include local wound care and potential consideration of transfusions. Additionally, in a new publication, the frequency of persistent ulcers was reduced in patients using voxelotor.

Vitamin D deficiency

Vitamin deficiency can be present in many patients with sickle cell disease. The most commonly reported one is vitamin D deficiency, with a deficiency or insufficiency prevalence of up to 90% in some cohort studies. Studies are currently attempting to understand the clinical implications of vitamin D deficiency, including its relationship to pain events and bone complications.

Treatment

Treatment of sickle cell disease includes general preventive and supportive measures, as well as treatment of specific complications. The NIH recently published "Evidence-based management of sickle cell disease expert panel report, 2014: guide to recommendations," which is an excellent resource for addressing the spectrum of treatment issues. Table 7-6 summarizes the results of major clinical trials influencing current clinical practice.

Preventive interventions

Patients with sickle cell disease, especially children, are at risk for life-threatening infections. Children should receive the pneumococcal vaccination, meningococcal vaccination, *Haemophilus influenzae* vaccination, and hepatitis B vaccination (please see Table 7-7 regarding the most recent guidelines). Additionally, children should have twice-daily penicillin prophylaxis at least until the age of 5 years. Vaccinations against influenza on an annual basis and the pneumococcal vaccine at 5-year intervals (after the childhood PCV-13 and PPV-23 vaccinations) should be administered to all patients. See Table 7-7 for a full vaccination summary list. Folic acid supplements are used by some to prevent depletion of folate stores and megaloblastic anemia related to chronic hemolysis, but this is probably unnecessary in industrial countries where diets are better and flour is fortified with folate.

Monitoring for end-organ disease is important for all patients with sickle cell disease, especially for those with sickle cell anemia. Screening for CNS abnormalities is important for patients with sickle cell anemia; annual stroke screen with TCD ultrasonography should be performed at least yearly for children of age 2 to 16 years with HbSS, $S\beta^0$-thalassemia, or other forms of sickle cell disease with a hemolytic phenotype. Patients with sickle cell anemia should receive screening MRI in early school age and again at least once as an adult (see further discussion of TCD in the sections "Central nervous system disease" and "RBC transfusion" in this chapter). For patients on chronic transfusion therapy to prevent or treat sickle cell complications, patients should be monitored for iron overload and consider annual hepatitis and HIV screenings. Ophthalmologic examinations should be performed periodically beginning around 10 years old. Patients with symptoms suggesting pulmonary hypertension, sleep-disordered breathing, or lung abnormalities should be evaluated with echocardiogram, polysomnography, or pulmonary function tests. Starting at the age of 10 years, annual screening for proteinuria is recommended. Additionally, routine age-appropriate screening and primary care should be continued to monitor for comorbidities unrelated to sickle cell disease. Genetic counseling services by trained individuals should be available for families with members that have sickle cell syndromes.

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Table 7-6 Important completed randomized clinical trials in sickle cell disease

Clinical trial	Year	Outcome
Penicillin Prophylaxis in Sickle Cell Disease (PROPS)	1986	Oral penicillin greatly reduces the incidence of invasive pneumococcal infections in children.
Penicillin Prophylaxis in Sickle Cell Disease II (PROPS II)	1995	Penicillin prophylaxis can be discontinued at 5 years old.
Multicenter Study of Hydroxyurea in Patients with Sickle Cell Anemia (MSH)	1995	Hydroxyurea reduces the frequency of painful episodes and appears to reduce the frequency of acute chest syndrome, transfusions, and hospitalizations.
National Preoperative Transfusion Study	1995	Simple transfusion to increase the hemoglobin concentration to 10 g/dL is as effective as exchange transfusion to reduce HbS to <30%.
Stroke Prevention Trial in Sickle Cell Anemia (STOP)	1998	First overt stroke can be prevented with red blood cell transfusions in high-risk children identified by TCD ultrasonography.
Optimizing Primary Stroke Prevention in Sickle Cell Anemia (STOP 2)	2005	Discontinuation of prophylactic red blood cell transfusions after 30 months results in a high rate of reversion to abnormal TCD velocities and stroke.
Hydroxyurea to Prevent Organ Damage in Very Young Children with Sickle Cell Anemia (BABY HUG)	2011	Hydroxyurea starting at 9 to 18 months of age did not prevent splenic and renal damage (the trial's primary endpoints), but it did decrease the frequency of dactylitis and painful episodes (secondary outcomes).
Stroke with Transfusions Changing to Hydroxyurea (SWiTCH)	2012	Terminated early because of futility for the primary composite endpoint of recurrent stroke and resolution of iron overload. There was an excess of recurrent strokes in the hydroxyurea arm ($N = 7$) compared with continued transfusions ($N = 0$).
Stroke (TWiTCH)	2016	For patients placed on blood transfusion therapy for elevated TCD velocities for at least 12 months, switching to maximum tolerated doses of hydroxyurea was noninferior to continued chronic blood transfusion therapy.
A Phase 3 Trial of L-Glutamine in Sickle Cell Disease	2017	L-Glutamine decreased crisis frequency from a median of 3.0 versus 4.0 compared to placebo. There were fewer hospitalizations (median 2.0 versus 3.0) and episodes of acute chest syndrome in the study drug arm compared to placebo.
Crizanlizumab for the Prevention of Pain Crises in Sickle Cell Disease	2017	Crizanlizumab, a P-selectin inhibitor, decreased crisis rate when compared to placebo (median of 1.63 in study arm versus 2.98 in placebo arm). Time to first crisis was also significantly longer in the crizanlizumab arm than the placebo arm (4.07 months versus 1.38 months).
A Phase 3 Randomized Trial of Voxelotor in Sickle Cell Disease	2019	Voxelotor, a HbS polymerization inhibitor, resulted in a higher percentage of patients achieve a Hb response of >1.0 g/dL than placebo (51% versus 7%) at week 24.

Table 7-7 Recommended immunizations for patients with sickle cell disease

Specific immunizations for patients with sickle cell disease	Frequency
Influenza vaccination	Annual
Pneumococcal vaccination	PPV-23: At least 2 doses 5 years apart, and then per some guidelines every 5 years after. If only 2 doses are given 5 years apart, a third dose should be given at age 65 years. PCV-13: 1 lifetime dose at age 18 or after
Meningococcal vaccination	Primary dosing depending on age at administration. Regardless of age, the patient should get a quadrivalent conjugate vaccine for at least 2 doses followed by a booster every 5 years. Additional dosing for serogroup B is recommended in some vaccine schedules.
Hepatitis B vaccine	Three doses at 0, 1, and 4 months. Subsequent frequency of antibody screening for continued immunization is not outlined.
Coronavirus vaccines	While frequency, dosing, and booster dosing remain unknown, effective vaccination is recommended for patients with sickle cell disease.

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RBC transfusion

The 2020 ASH guidelines for the management of sickle cell disease addresses the appropriate indications for transfusion of red blood cells. Patients with sickle cell disease often receive transfusions outside of approved indications, which may be unnecessary. Some indications for transfusion include moderate to severe acute splenic sequestration, symptomatic aplastic crisis, cerebrovascular accident (occlusive or hemorrhagic), acute ocular vaso-occlusive events, and acute chest syndrome with hypoxemia. Although the first 2 events only require correction of anemia and thus are treated with simple transfusion, stroke, ocular events, and severe acute chest syndrome are best treated with exchange transfusion aimed at decreasing the percentage of HbS to <30% and increasing the Hb level to 9 to 10 g/dL. In addition, transfusions are indicated for the prevention of recurrent strokes as well as for the treatment of high-output cardiac failure. As mentioned, a stroke screen with an abnormal TCD velocity can identify children with HbSS and $S\beta^0$ at high risk of primary overt stroke, which can be prevented by chronic transfusion therapy. Transfusion has also been advocated for patients with severe pulmonary hypertension and chronic nonhealing leg ulcers and to prevent recurrences of priapism, but clinical trial data are lacking. When chronic transfusion is indicated, RBCs may be administered as a partial exchange transfusion, which may offer a long-term advantage of delaying iron accumulation. The goal of chronic transfusion is usually to achieve a nadir total Hb level of 9 to 10 g/dL with the HbS under 30% to 50%. It is important to avoid the hyperviscosity associated with Hb levels >11 to 12 g/dL in the presence of 30% or more HbS. Patients with HbSC requiring transfusion pose special challenges, with the need to avoid hyperviscosity usually necessitating exchange transfusion (goal HbA >70%) to ensure the Hb concentration does not exceed 11 to 12 g/dL.

Preoperative transfusion in preparation for surgery under general anesthesia may afford protection against perioperative complications and death but is probably not indicated in all cases, particularly minor procedures in children. In a multicenter study, simple transfusion to a total Hb level of 10 g/dL afforded protection equal to partial exchange and was associated with less red cell alloimmunization. Another randomized trial, TAPS Study, included patients with sickle cell anemia undergoing low- or medium-risk surgery. Subjects were randomized to either preoperative transfusion or no transfusion. Of 33 patients in the no-preoperative-transfusion group, 39% had clinically important complications, compared with 15% in the preoperative-transfusion group ($P = 0.023$), leading to early termination because of the number of complications in the nontransfusion arm. Patients undergoing prolonged surgery or with regional compromise of blood supply (eg, during orthopedic surgery), hypothermia, or a history of pulmonary or cardiac disease may do better with preoperative exchange transfusion, although this has not been evaluated in a randomized clinical trial. Transfusions also may be useful for some patients preparing for intravenous ionic contrast studies, dealing with chronic intractable pain, or facing complicated pregnancy. Transfusions are not indicated for the treatment of steady-state anemia, uncomplicated pain events, uncomplicated pregnancy, most leg ulcers, or minor surgery not requiring general anesthesia.

A few complications of chronic transfusion therapy are worth noting. Up to 30% of patients with sickle cell disease who repeatedly undergo transfusion become alloimmunized to RBC antigens (especially E, C, and Kell), and this risk increases with increasing exposure. Alloimmunization predisposes patients to delayed transfusion reactions and possible hyperhemolysis, which can lead to potentially life-threatening anemia and multiorgan failure. Severe painful crises with a decrease in the Hb level within days to weeks of a transfusion should alert the clinician to consider this diagnosis. Identification of a new alloantibody may not be made acutely, and reticulocytopenia can be an associated finding. In this situation, additional transfusions are hazardous and should be avoided if at all possible and clinicians could consider immunosuppressive therapy (intravenous immunoglobulin, steroids, rituximab, and/or eculizumab). The current ASH guidelines for transfusion support attempted to address alloimmunization with 2 evidence-based guideline statements. First, the panel suggests an extended red cell antigen profile by genotype or serology over only ABO/RhD typing at the earliest opportunity. Second, the guideline panel recommends prophylactic red cell antigen matching for Rh (C, E, or C/c, E/e) and K antigens over only ABO/RhD matching for patients with SCD.

Second, patients with sickle cell disease who receive frequent transfusions are at increased risk for the development of iron overload. Patients with frequent transfusions should be screened for iron overload with MRI for liver iron content annually or every other year. If a patient has significant evidence of cardiac dysfunction while on chronic transfusion, T2* MRI for cardiac liver iron could be obtained. As discussed in the "Thalassemia" section, patients with evidence of iron overload benefit from iron chelation. Patients with evidence of iron overload benefit from iron chelation. The types and properties of iron chelators are described in the thalassemia section and Table 7-2.

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Modifying the disease course

In addition to chronic transfusions, other disease-modifying treatments are currently available: (1) hydroxyurea, (2) L-glutamine, (3) crizanlizumab, and (4) voxelotor, which are ameliorative; (5) hematopoietic stem cell transplantation, which is curative; and (6) gene therapy, which is currently being considered as possibly ameliorative versus curative.

Hydroxyurea

Based on the knowledge that patients with high HbF levels have less severe disease, many investigators tested a variety of experimental strategies for pharmacologic induction of HbF production and identified hydroxyurea as efficacious and practical. A multicenter, randomized, placebo-controlled trial then found that daily oral administration of hydroxyurea significantly reduced the frequency of pain episodes, acute chest syndrome, and transfusions in adult HbSS patients (Multicenter Study of Hydroxyurea in Patients with Sickle Cell Anemia [MSH] study). No serious short-term adverse effects were observed, although monitoring of blood counts was required to avoid potentially significant cytopenias. Interestingly, the therapeutic response to hydroxyurea sometimes precedes or occurs in the absence of a change in HbF levels, suggesting that a reduction in white blood cell count and other mechanisms may be beneficial. Laboratory studies revealed that hydroxyurea reduced adherence of RBCs to vascular endothelium, improved RBC hydration, and increased the time to polymerization. Follow-up at 17.5 years indicates that patients taking hydroxyurea seem to have reduced mortality without evidence for an increased incidence of malignancy. Classical indications for hydroxyurea include frequent painful episodes, recurrent acute chest syndrome, severe symptomatic anemia, and other severe vaso-occlusive events. Given the safety of hydroxyurea and that HbSS is a morbid condition, many clinicians use hydroxyurea more liberally even when the classical indications for hydroxyurea therapy are not present. There are now guideline recommendations to strongly consider the use of hydroxyurea in patients with sickle cell anemia who have daily chronic pain that interferes with quality of life. Clinical trials of hydroxyurea in children also show a reduction in the frequency of painful episodes, but no convincing evidence yet indicates that early hydroxyurea therapy prevents or delays the onset of organ damage. Pregnancy should be avoided while taking hydroxyurea. Hydroxyurea should still be considered first-line therapy in patients who meet the guideline recommendations for treatment.

L-Glutamine

The role of oxidative stress in the pathophysiology of sickle cell disease is complex. The integrity of the red blood cell membrane is affected by reactive oxygen species generation, with a dose-dependent effect of reactive oxygen species on membrane rigidity and decreased elasticity. Both red cell and leukocyte adhesion have been shown to increase with superoxide production in sickle cell disease. L-Glutamine therapy, which increases the proportion of reduced nicotinamide adenine dinucleotide in sickle red blood cells and presumably reduces oxidative stress and potentially painful events, was tested in a randomized controlled clinical trial. The randomized study included 230 patients (age 5 to 58 years) with either HbSS or $S\beta^0$-thalassamia with a history of 2 or more crises during the previous year. Patients randomized to L-glutamine had significantly fewer sickle cell crises than patients receiving placebo (median 3.0 versus 4.0 crises). Fewer hospitalizations (median 2.0 versus 3.0) and episodes of acute chest syndrome occurred in the study group. The majority of subjects on both arms were on hydroxyurea. L-Glutamine (Endari) was approved by the United States Food and Drug Administration (FDA) in 2017. Endari is available in powder form and is mixed with room temperature food at doses of 5 to 15 grams based on weight, and given twice daily.

Selectin inhibition

Currently, the only FDA-approved therapy in this area is crizanlizumab, an intravenous P-selectin inhibitor. This was evaluated for prevention of vaso-occlusive crises. The monthly infusion was noted to decrease crisis rate when compared with placebo (median of 1.6 in study arm versus 3.0 in placebo). Subjects using crizanlizumab also had a prolonged time to the first crisis that was significantly longer than those on placebo (4.07 versus 1.38 months). Side effects of therapy were primarily related to infusion related pain as well as rare anaphylactic reactions, which were reported to range from mild to severe.

Although the phase 2 trial of rivipansel, a pan-selectin inhibitor, was promising, a subsequent phase 3 study was not conclusive.

Antisickling

Voxelotor is an oral therapy that modifies Hb to increase affinity between Hb and oxygen. The modified Hb is thought to prevent polymerization and thereby sickling of HbS. In the pivotal phase 3 GBT-HOPE trial, voxelotor was found to increase hemoglobin and also reduce markers of chronic hemolysis. While subsequent abstracts

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report improvement in pain episodes, recent publications failed to show a consistent improvement in pain with voxelotor therapy. Side effects of therapy include gastrointestinal complaints that are reported to respond to dose reductions.

Hematopoietic stem cell transplantation

Allogeneic transplantation is curative therapy for people with sickle cell disease. In a recent assessment of outcomes from 3 transplant registries that included 1000 recipients of HLA-identical sibling transplants performed between 1986 and 2013, the 5-year overall survival for children under 16 years was 95%, with an event-free survival of 93%. For those over the age of 15 years, the overall survival and event-free survival were both 81%. In most centers, few patients meet the usual eligibility criteria, which includes an HLA-matched sibling donor. Questions remain about who should be referred for transplant and whether asymptomatic people with severe genotypes should be sent for transplant. Results from a Belgian registry found that patients treated with hydroxyurea had improved survival compared to those who underwent transplant. In that study with 15 years of follow-up, mortality rates for hydroxyurea and transplant groups were 0.14 and 0.36 per 100 patient-years, respectively. Longer follow-up might change these numbers and survival is not the only outcome to assess the risk-benefit profile of transplant. Alternative sources such as umbilical cord blood, unrelated matched, and haplotype donors are now being investigated. These alternative donor options and nonmyeloablative conditioning regimens have shown some promise, but remain investigational. As these efforts are undergoing further development, consideration of long-term effects of transplant such as loss of fertility and secondary malignancies should also be considered.

Gene therapy

Recent studies show improvement and potential amelioration of progression of sickle cell disease with the use of gene therapy. Gene therapy represents a heterogeneous group of treatments that all aim to correct the underlying genetic defect in sickle disease. Integration of the functional gene is currently in trials via viral vector, clustered regularly interspaced short palindromic repeats (CRISPR) technology, or direct point mutation correction. While these therapies offer potential promise, the current data in the duration of response and magnitude of response are still pending. Additionally, the optimal method of bone marrow collection as well as the optimal chemotherapy based preparatory regimens are also being studied.

CLINICAL CASE *(continued)*

The case in this section describes a patient with sickle cell anemia who previously experienced pain episodes without major complications related to his disease. He is admitted for a pain crisis, and multiorgan failure ensues. Acute multiorgan failure is a well-described complication of sickle cell disease. High baseline hemoglobin levels may represent a key risk factor. Acute multiorgan failure is often precipitated by a severe acute pain crisis, and is thought to be secondary to widespread intravascular sickling, fat embolization, and subsequent ischemia within affected organs. Aggressive transfusion therapy can be lifesaving and result in complete recovery.

KEY POINTS

- The clinical manifestations of sickle cell disease are primarily caused by hemolysis and vaso-occlusion.
- Multiple cellular and genetic factors contribute to the phenotypic heterogeneity of sickle cell disease.
- The hemoglobin F level is a major determinant of clinical manifestations and outcomes.
- Pneumococcal sepsis in high-resource countries is now uncommon, but it remains a potential cause of death in infants and young children, particularly in low-resource countries. Universal newborn screening, compliance with penicillin prophylaxis, and vaccination remain a priority.
- Human parvovirus infection is one of the major viruses that cause aplastic crisis.
- Splenic sequestration should be considered in the differential diagnosis of a sudden marked decrease in the hemoglobin concentration.
- There are differences in frequency of clinical events and survival among the various genotypes of sickle cell disease.
- Sickle cell disease is a leading cause of stroke in young individuals, and a substantial incidence of covert or silent infarctions has recently been appreciated.
- A randomized clinical trial has demonstrated efficacy of red cell transfusion in preventing first stroke in children with abnormal TCD velocity.
- A randomized clinical trial demonstrated that preoperative simple transfusion was as effective as exchange transfusion. The preoperative management of the older patient, particularly with cardiac or pulmonary dysfunction, has not been defined.

Key Points continue

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KEY POINTS *(continued)*

- A randomized, placebo-controlled clinical trial has established the efficacy of hydroxyurea in reducing the frequency of painful episodes and acute chest syndrome. A follow-up study suggests a reduction in mortality for patients taking hydroxyurea.
- The causes of acute chest syndrome include infection, fat embolism, and pulmonary infarction.

Other hemoglobinopathies

Hemoglobin E

HbE is a β-chain variant with highest frequency in Southeast Asia. The highest prevalence occurs in Myanmar and Thailand, where the gene frequency may approach 70% in certain regions. The gene frequency is also high in Laos, Cambodia, and Vietnam. It is also found in Sri Lanka, northeastern India, Nepal, Bangladesh, Malaysia, Indonesia, and the Philippines. It has become more common in the United States during the past 20 to 30 years as a result of immigration. The structural change is a substitution of glutamic acid by lysine at the 26th position of the β-globin chain. The mutation is also thalassemic because the single-base GAG/AAG substitution creates a cryptic splicing site, which results in abnormal mRNA processing and reduction of mRNA that can be translated. HbE is also slightly unstable in the face of oxidant stress and is sometimes referred to as a *thalassemic hemoglobinopathy.*

Individuals with hemoglobin E trait are asymptomatic with or without mild anemia (hemoglobin >12 g/dL), and mild microcytosis. Peripheral smear may be normal or may show hypochromia, microcytosis, target cells, irregularly contracted cells, and basophilic stippling. HbE usually makes up 30% or less of total hemoglobin. The HbE concentration is lower with the coinheritance of α-thalassemia. Homozygotes (HbE disease) are usually asymptomatic with no overt hemolysis or splenomegaly. Individuals may have mild anemia, microcytosis (MCV approximately 65 to 69 fL in adults and 55 to 65 fL in children), and reduced MCH. Peripheral smear shows hypochromia, microcytosis, and a variable number of target cells and irregularly contracted cells. HbE plus HbA_2 makes up 85% to 99% of the total hemoglobin. The compound heterozygous state, HbE β-thalassemia, results in a very variable phenotype ranging from thalassemia trait, NTDT, to TDT, depending on the β-mutation. It is now one of the more common forms of thalassemia in the United States. It is characterized by microcytic anemia, with mildly increased reticulocytosis. The peripheral smear includes anisocytosis, poikilocytosis, hypochromia, microcytosis, target cells, nucleated red blood cells, and irregularly contracted cells. HbE β^0-thalassemia is associated with a mostly HbE electrophoretic pattern, with increased amounts of HbF and HbA_2. The electrophoretic pattern in HbE β^+-thalassemia is similar except for the presence of approximately 15% HbA. HbE comigrates with HbC and HbA_2 on cellulose acetate electrophoresis and isoelectric focusing. HPLC separates HbC, HbA_2, and HbE.

Patients with HbE disease are usually asymptomatic and do not require specific therapy. However, patients with coinherited HbE and β-thalassemia, especially those with HbE-β^0, may have significant anemia. Some need intermittent or chronic RBC transfusions, and some may benefit from splenectomy.

Hemoglobin C

HbC is the third most common mutant hemoglobin, after HbS and HbE. The HbC mutation arose in West Africa. The prevalence in African Americans is 2% to 3%. The hemoglobin mutant results from the substitution of lysine for glutamic acid as the sixth amino acid of β-globin, the consequence of a single nucleotide substitution (GAG/AAG) in the sixth codon. The resultant positive-to-negative charge difference on the surface of the HbC tetramer results in a molecule with decreased solubility in both the oxy and deoxy forms, which may undergo intraerythrocytic aggregation and crystal formation. HbC stimulates the K:Cl cotransport system, promoting water loss and resulting in dehydration and poorly deformable RBCs that have a predilection for entrapment within the spleen. Consequently, patients with HbCC and patients with HbC β-thalassemia have mild chronic hemolytic anemia and splenomegaly. Patients may develop cholelithiasis, and the anemia may be more exaggerated in association with infections. Heterozygous individuals (HbC trait) are clinically normal; however, identifying the diagnosis is important for genetic counseling. The coinheritance of HbS and HbC results in a form of sickle cell disease, HbSC (see the section "Sickle cell disease" in this chapter).

Laboratory studies in HbCC show a mild hemolytic anemia, microcytosis, and slightly elevated reticulocyte counts. The MCHC is elevated because of the effect of HbC on cellular hydration. The peripheral blood smear shows prominent target cells, microcytosis, and irregularly contracted red cells. RBCs containing hemoglobin crystals also may be seen on the blood smear, particularly in patients who have had splenectomy. Individuals with HbC trait have normal hemoglobin levels, and microcytosis is common. The peripheral smear may be normal or may show microcytosis and target cells. Confirmation of the

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diagnosis requires identification of HbC, which comigrates with HbA_2, HbE, and HbO^{Arab} on cellulose acetate electrophoresis and isoelectric focusing. Thus, HbC must be distinguished by citrate gel electrophoresis or HPLC. Specific treatment for patients with HbCC is not generally necessary.

Hemoglobin D

HbD is usually diagnosed incidentally. HbD^{Punjab} (also called $HbD^{Los\ Angeles}$) results from the substitution of glutamine for glutamic acid at the 121st position of the β-chain. This mutant has a prevalence of approximately 3% in the Northwest Punjab region of India, but is also encountered in other parts of the world. Patients who are homozygous (HbDD) may have a mild hemolytic anemia. Individuals who are heterozygous (HbAD) are clinically normal, with normal blood counts and a peripheral smear with the occasional target cells. The major clinical relevance of HbD is with compound heterozygous inheritance with HbS, resulting in a form of sickle cell disease, perhaps as a result of the low affinity of HbD promoting hemoglobin deoxygenation. The diagnosis of HbAD (D trait) or HbDD is made by hemoglobin electrophoresis. HbS and HbD have similar electrophoretic mobility on alkaline cellulose acetate electrophoresis and isoelectric focusing. They can be differentiated by acid citrate agar electrophoresis, HPLC, or solubility studies. This distinction is important for genetic and prognostic counseling.

KEY POINTS

- Hemoglobins C, D, and E are common hemoglobin variants that can have significant consequences when coinherited with hemoglobin S.
- Homozygosity for hemoglobin E (EE) is a mild condition, but compound heterozygosity for HbE and β-thalassemia can be a clinically significant thalassemia syndrome.

Unstable hemoglobin

Unstable hemoglobin variants are inherited in an autosomal dominant pattern, and affected individuals are usually heterozygotes. Unstable hemoglobins constitute one of the largest groups of hemoglobin variants, although individually, each is rare. In both Hb Köln (β_{98} Val/Met substitution) and in Hb Zurich (β_{63} His/Arg), the amino acid substitution destabilizes the heme pocket. Other mechanisms that destabilize hemoglobin include (1) alteration of the $\alpha_1\beta_1$ interface region (eg, Hb Tacoma, β_{30} Arg/Ser), (2) distortion of the helical configuration of structurally important regions (eg, Hb Bibba, α_{136} Leu/Pro), and (3) introduction of the interior polar amino acid (eg, Hb Bristol, β_{67} Val/Asp). Unstable γ-chain variants (eg, Hb Poole, γ_{130} Trp/Gly) can cause transient hemolytic anemia in the neonate that spontaneously resolves.

These abnormal hemoglobins precipitate spontaneously or with oxidative stress. Precipitated hemoglobin inclusions (Heinz bodies seen using a supravital stain) impair erythrocyte deformability, resulting in premature erythrocyte destruction by macrophages of the liver and spleen. The severity of the hemolysis varies with the nature of the mutation but may be accelerated by fever or ingestion of oxidant drugs.

An unstable hemoglobinopathy should be suspected in a patient with hereditary nonspherocytic hemolytic anemia. The hemoglobin level may be normal or decreased. Hypochromia of the RBCs (resulting from loss of hemoglobin because of denaturation and subsequent pitting), "bite cells," and basophilic stippling may occur. The evaluation includes hemoglobin electrophoresis (which is often normal), crystal violet Heinz-body staining, and the isopropanol stability test. The isopropanol test may be falsely positive in the neonate because of high fetal hemoglobin levels, so the heat stability test should be used during the first months of life. Management includes avoidance of oxidant agents, and some recommend supplementation with folic acid. Splenectomy may be useful for patients with severe hemolysis and splenomegaly. The risk of thrombosis is high after splenectomy in individuals with a severely unstable hemoglobin, and thus patients should be educated and closely evaluated in this regard.

Some unstable hemoglobins may also have altered oxygen affinity, which could exacerbate (decreased oxygen affinity) or ameliorate (increased oxygen affinity) the degree of anemia.

Methemoglobinemia

Methemoglobinemia is characterized by a decrease in hemoglobin's oxygen-carrying capacity because of oxidation of the iron moieties in hemoglobin from ferrous (Fe^{2+}) to ferric (Fe^{3+}), which is unable to bind and transport oxygen. High methemoglobin levels cause a functional anemia. Methemoglobinemia can result from either congenital or acquired processes. Congenital forms are caused by (1) autosomal dominant mutations in α- or β-globin chains, producing variants collectively called *hemoglobin M*, or (2) autosomal recessive defects in the enzyme cytochrome b_5 reductase (CYB5R). Acquired

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methemoglobinemia is much more common and is caused by exposure to substances that cause oxidation of hemoglobin, including direct oxidizing agents (eg, benzocaine), indirect oxidants (eg, nitrites), or metabolic activation (eg, dapsone). Methemoglobinemia should be considered in the setting of dyspnea, cyanosis, and hypoxemia that is refractory to supplemental oxygen. The clinical presentation is variable depending on the percentage of methemoglobin, rate of methemoglobin accumulation, rate of clearance, and magnitude of exposure. The clinical spectrum includes cyanosis, pallor, weakness, fatigue, headache, metabolic acidosis, dysrhythmias, seizures, central nervous system depression, coma, and death. Generally, the higher the methemoglobin level, the more severe the clinical symptoms. Clinical evaluation for refractory hypoxemia, chocolate-colored blood, arterial or venous blood gas with cooximetry, and determination of methemoglobin percentage are key clues. Treatment for acquired methemoglobinemia generally includes removal of the inciting agent, use of methylene blue, and high-flow oxygen to enhance natural degradation of methemoglobin.

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